EORM-PTO-1390 (Rev. 12-29-99)

DEPARTMENT OF COMMI-TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

001560-397 U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5) To be a Qian of 830

INTERNATIONAL APPLICATION NO. PCT/JP00/05722

INTERNATIONAL FILING DATE 24 August 2000

PRIORITY DATE CLAIMED 24 August 1999

TITLE OF INVENTION

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GENES ENCODING PROTEINS REGULATING THE pH OF VACUOLES APPLICANT(S) FOR DO/EO/US

Shigeru IIDA, Sachiko TANAKA, and Yoshishige INAGAKI

| Applican | t herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information: |
|----------|---|
| 1 🛛 | This is a FIRST submission of items concerning a filing under 35 U.S.C. 371. |

This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.

This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and the PCT Articles 22 and 39(1).

-AIT IN SHADEMARK OFFICE

A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

A copy of the International Application as filed (35 U.S.C. 371(c)(2))

is transmitted herewith (required only if not transmitted by the International Bureau).

has been transmitted by the International Bureau.

is not required, as the application was filed in the United States Receiving Office (RO/US)

6. A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7 1110 Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))

are transmitted herewith (required only if not transmitted by the International Bureau).

have been transmitted by the International Bureau.

have not been made; however, the time limit for making such amendments has NOT expired.

have not been made and will not be made.

8 = 0 A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5))

Items 11, to 16, below concern other document(s) or information included:

An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

A FIRST preliminary amendment.

A SECOND or SUBSEQUENT preliminary amendment.

14. A substitute specification.

A change of power of attorney and/or address letter.

16. Other items or information:

International Search Report

Sequence Listing (paper copy) Japanese PCT Request Form

PCT Notice Informing the Applicant of the Communication of the International Application to the Designated Offices (Form PCT/IB/308) Cover page from published PCT international application (WO 01/14560)

| J.S. APPLICATION NO. III kn | TY 7 8 3 (123 | PCT/JF 2/05722 | N NO. | | | EY'S DOCKET NUMBER 60-397 | |
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| , o bo acception | | | | CAL | CULATIONS | PTO USE ONLY | |
| 7. The following fees are submitted: | | | | | | | |
| Asic National Fee (37 CFR 1.492(a)(1)-(5)): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$1,000.00 (960) | | | | | | | |
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| Surcharge of \$130.00 (154) for furnishing the oath or declaration later than 20 30 30 months from the earliest claimed priority date (37 CFR 1.492(e)). | | | | | | | |
| Claims | Number Filed | Number Extra | Rate | | | | |
| otal Claims | 51 -20 = | 31 | X\$18.00 (966) | \$ | 558.00 | | |
| dependent Claims | 3 -3 = | 0 | X\$80.00 (964) | \$ | | | |
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| Reduction for 1/2 for filling by small entity, if applicable (see below). | | | | | | - | |
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| TOTAL NATIONAL FEE = | | | | \$ | 1,418.00 | | |
| - 1 U Fee för recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 (581) per property + | | | | | 40.00 | | |
| Fall | | TOTAL FE | S ENCLOSED = | \$ | 1,458.00 | | |
| | | | | | Amount to be: refunded | \$ | |
| | | | | | charged | \$ | |
| a. Small entit | y status is hereby claimed. | | | | | | |
| | the amount of \$ 1,458.00 | | | | | | |
| is enclosed | Please charge my Deposit Account No. <u>02-4800</u> in the amount of \$ to cover the above fees. A duplicate copy of this sheet is enclosed. | | | | | | |
| d. The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-4800. A duplicate copy of this sheet is enclosed. | | | | | | | |
| NOTE: Where a must be filed an | n appropriate time limit under d granted to restore the applic | 37 CFR 1.494 or 1.495 has r ation to pending status. | ot been met, a petitio | n to r | evíve (37 CFR 1 | .137(a) or (b)) | |
| SEND ALL CORRESPONDENCE TO: | | | | | | | |
| Ronald L Burns, D P.O. Box | Grudziecki Joane, Swecker & MATHI: < 1404 | / 0 | NATURE | | · / | | |
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| April 24, 2001 REGISTRATION NUMBER | | | | | | | |

Patent Attorney's Docket No. <u>001560-397</u>

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| In re Patent Application of |) |
|---|----------------------------------|
| Shigeru IIDA et al |) Group Art Unit: To be assigned |
| Application No.: To be assigned (National Stage of PCT International Appln. No. PCT/JP00/05722 filed August 24, 2000) | Examiner: To be assigned) |
| Filed: April 24, 2001 |) |
| For: GENES ENCODING PROTEINS REGULATING THE pH OF VACUOLES |))) |

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents Washington, D.C. 20231

Sir:

Prior to examination of the above-identified application on the merits, please amend the application as follows:

IN THE SPECIFICATION

Kindly replace the paragraph beginning at page 5, line 15, with the following:

-- The present invention also provides a plant in which said gene or said vector has been introduced or a progeny thereof having the same property as said plant, or a tissue thereof.--

Kindly replace the paragraph beginning at page 5, line 19, with the following:

-- The present invention also provides a cut flower of the above plant or a progeny thereof.--

Please add the paper copy of the Sequence Listing included herewith to the application, after page 19 and before the Claims on page 20.

Please renumber the pages accordingly.

IN THE CLAIMS

Please replace claims 7, 9, and 11-14 as follows:

- 7. (Amended) A vector comprising the gene according to claim 1.
- 9. (Amended) A protein encoded by the gene according to claim 1.
- 11. (Amended) A plant in which the gene according to claim 1 has been introduced or a progeny thereof having the same property as said plant, or a tissue thereof.
- 12. (Amended) A cut flower of the plant according to claim 11 or a progeny thereof having the same property as said plant.
- 13. (Amended) A method of regulating the pH of vacuoles comprising introducing the gene according to claim 1 into a plant or plant cells and then allowing said gene to be expressed in said plant or plant cells.
- 14. (Amended) A method of controlling the flower color of a plant comprising introducing the gene according to claim 1 into a plant or plant cells and then allowing said gene to be expressed in said plant or plant cells.

Please add new claims 15-51 as follows:

- -15. A vector comprising the gene according to claim 2.
- 16. A vector comprising the gene according to claim 3.
- 17. A vector comprising the gene according to claim 5.
- 18. A vector comprising the gene according to claim 6.
- 19. A host cell transformed with the vector according to claim 15.
- 20. A host cell transformed with the vector according to claim 16.
- 21. A host cell transformed with the vector according to claim 17.
- 22. A host cell transformed with the vector according to claim 18.
- 23. A protein encoded by the gene according to claim 2.
- 24. A protein encoded by the gene according to claim 3.
 - 25. A protein encoded by the gene according to claim 5.
 - 26. A protein encoded by the gene according to claim 6.
- 27. A method of producing a protein that has an activity of regulating the pH of vacuoles, said method comprising culturing or growing the host cell according to claim 19 and then harvesting said protein from said host cell.
- 28. A method of producing a protein that has an activity of regulating the pH of vacuoles, said method comprising culturing or growing the host cell according to claim 20 and then harvesting said protein from said host cell.

- 29. A method of producing a protein that has an activity of regulating the pH of vacuoles, said method comprising culturing or growing the host cell according to claim 21 and then harvesting said protein from said host cell.
- 30. A method of producing a protein that has an activity of regulating the pH of vacuoles, said method comprising culturing or growing the host cell according to claim 22 and then harvesting said protein from said host cell.
- 31. A plant in which the gene according to claim 2 has been introduced or a progeny thereof having the same property as said plant, or a tissue thereof.
- 32. A plant in which the gene according to claim 3 has been introduced or a progeny thereof having the same property as said plant, or a tissue thereof.
- 33. A plant in which the gene according to claim 5 has been introduced or a progeny thereof having the same property as said plant, or a tissue thereof.
- 34. A plant in which the gene according to claim 6 has been introduced or a progeny thereof having the same property as said plant, or a tissue thereof.
- 35. A cut flower of the plant according to claim 31 or a progeny thereof having the same property as said plant.
- 36. A cut flower of the plant according to claim 32 or a progeny thereof having the same property as said plant.
- 37. A cut flower of the plant according to claim 33 or a progeny thereof having the same property as said plant.

- 38. A cut flower of the plant according to claim 34 or a progeny thereof having the same property as said plant.
- 39. A method of regulating the pH of vacuoles comprising introducing the gene according to claim 2 into a plant or plant cells and then allowing said gene to be expressed in said plant or plant cells.
- 40. A method of regulating the pH of vacuoles comprising introducing the gene according to claim 3 into a plant or plant cells and then allowing said gene to be expressed in said plant or plant cells.
- 41. A method of regulating the pH of vacuoles comprising introducing the gene according to claim 5 into a plant or plant cells and then allowing said gene to be expressed in said plant or plant cells.
- 42. A method of regulating the pH of vacuoles comprising introducing the gene according to claim 6 into a plant or plant cells and then allowing said gene to be expressed in said plant or plant cells.
- 43. A method of controlling the flower color of a plant comprising introducing the gene according to claim 2 into a plant or plant cells and then allowing said gene to be expressed in said plant or plant cells.
- 44. A method of controlling the flower color of a plant comprising introducing the gene according to claim 3 into a plant or plant cells and then allowing said gene to be expressed in said plant or plant cells.

- 45. A method of controlling the flower color of a plant comprising introducing the gene according to claim 5 into a plant or plant cells and then allowing said gene to be expressed in said plant or plant cells.
- 46. A method of controlling the flower color of a plant comprising introducing the gene according to claim 6 into a plant or plant cells and then allowing said gene to be expressed in said plant or plant cells.
- 47. A method of controlling the flower color of a plant comprising suppressing expression of the gene according to claim 1 in a plant or plant cells.
- 48. A method of controlling the flower color of a plant comprising suppressing expression of the gene according to claim 2 in a plant or plant cells.
- 49. A method of controlling the flower color of a plant comprising suppressing expression of the gene according to claim 3 in a plant or plant cells.
- 50. A method of controlling the flower color of a plant comprising suppressing expression of the gene according to claim 5 in a plant or plant cells.
- 51. A method of controlling the flower color of a plant comprising suppressing expression of the gene according to claim 6 in a plant or plant cells.—

REMARKS

Prior to examination, entry of the foregoing is respectfully requested.

Claims 7, 9, and 11-14 have been amended simply to delete multiple dependencies in the claims and correct claim dependencies. Minor amendments relating to matters of form only have also been made.

New claims 15-51 have been added, directed to preferred embodiments of the invention in view of the deletion of multiple dependent claims. Support for these additional claims may be found at the very least in original claims 1-14 and at page 19, lines 12-24. No new matter has been added.

In the event that there are any questions relating to this Preliminary Amendment, or to the application in general, it would be appreciated if the Examiner would telephone the undersigned attorney at (508) 339-3684 concerning such questions so that prosecution of this application may be expedited.

Early and favorable action in the form of a Notice of Allowance is respectfully requested and believed to be in order.

Respectfully submitted,

Donna M. Meuth

Registration No. 36,607

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

P.O. Box 1404 Alexandria, Virginia 22313-1404

(703) 836-6620

Date: April 24, 2001

(11/00)

Attachment to Preliminary Amendment dated April 24, 2001

Marked-up Copy

Page 5, Paragraph Beginning at Line 15

The present invention also provides a plant in which said gene or said vector has been introduced or [an] a progeny thereof having the same property as said plant, or a tissue thereof.

Attachment to Preliminary Amendment dated April 24, 2001

Marked-up Copy

Page 5, Paragraph Beginning at Line 19

The present invention also provides a cut flower of the above plant or [an] \underline{a} progeny thereof.

Attachment to Preliminary Amendment dated April 24, 2001

Marked-up Claims 7, 9, and 11-14

- (Amended) A vector comprising the gene according to <u>claim 1</u> [any one of the claims 1 to 6].
- (Amended) A protein encoded by the gene according to <u>claim 1</u> [any one of claims 1 to 6].
- 11. (Amended) A plant in which the gene according to claim 1 [any one of claims 1 to 6 or the vector according to claim 7] has been introduced or a [an] progeny thereof having the same property as said plant, or a tissue thereof.
- 12. (Amended) A cut flower of the plant according to claim 11 or a [an] progeny thereof having the same property as said plant.
- 13. (Amended) A method of regulating the pH of vacuoles comprising introducing the gene according to <u>claim 1</u> [any one of claims 1 to 6 or the vector according to claim 7] into a plant or plant cells and then allowing said gene to be expressed <u>in said</u> plant or plant cells.
- 14. (Amended) A method of controlling the flower color of a plant [plants] comprising introducing the gene according to claim 1 [any one of claims 1 to 6 or the vector according to claim 7] into a plant or plant cells and then allowing said gene to be expressed in said plant or plant cells.

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DESCRIPTION

GENES ENCODING PROTEINS REGULATING THE PH OF VACUOLES

5 Technical Field

The present invention relates to genes encoding proteins that regulate the pH of vacuoles, and the uses thereof.

10 Background Art

In the flower industry, the development of novel or varied cultivars of flowering plants is important, and flower color is one of the most important traits of flowers. Although cultivars of various colors have been bred using conventional breeding by crossing, it is rare that a single plant species has cultivars of all colors. Thus, there is a need for the development of cultivars having a variety of colors.

The main components of flower color are a group of flavonoid compounds termed anthocyanins. It is known that a variety of anthocyanins occur in plants, and the structure of many of them have already been determined. The color of anthocyanins depends partly on their structures. Progress has been made in the study on the enzymes and genes involved in the biosynthesis of anthocyanins, and in some studies molecular biological techniques and gene introductions into plants were used to change the structure of anthocyanins, leading to changes in the color of flowers (Holton and Cornish, Plant Cell, 7:1071 (1995); Tanaka et al., Plant Cell Physiol. 39:1119 (1998)). The color of anthocyanins also depends on the pH of the aqueous solution, and the same anthocyanin may appear blue when the pH of the aqueous solution is neutral to weakly alkaline (Saito and Honda, Genda Kadaku (Chemistry Today), May 1998, pp. 25).

It is also known that since anthocyanins are present in the vacuole of the cell, the pH of vacuoles has a

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great impact on the color of flowers (Holton and Cornish, Plant Cell, 7 (1995); Mol et al., Trends Plant Sci. 3:212 (1998)). For example, in morning glory (Ipomea tricolor), it is known that the reason why red-purple buds bloom into blue flowers is that the pH of vacuoles in petal epithelium rises from 6.6 to 7.7 (Yoshida et al., Nature 373:291 (1995)).

It is thought that the vacuole of plant cells is regulated by vacuolar proton-transporting ATPase and vacuolar proton-transporting pyrophosphatase (Leigh et al., The Plant Vacuole (1997), Academic Press), but the mechanism of how these proton pumps are involved in the color of flowers has not been elucidated. It was also known that a sodium ion-proton antiporter (hereinafter referred to as Na⁺-H⁺ antiporter) exits in plant vacuoles and that the Na⁺-H⁺ antiporter transports sodium ions into vacuoles, depending on the proton concentration gradient between the outside and the inside of vacuoles, whereupon protons are transported outside of vacuoles resulting a reduced proton concentration gradient.

Furthermore, the Na⁺-H⁺ antiporter is thought to be a protein with a molecular weight of about 170,000. However, there are many unknown factors involved in the regulation of pH of vacuoles, and the mechanism of regulating the pH of vacuoles, in particular the petal vacuoles, is uncertain (Leigh et al., The Plant Vacuole (1997), Academic Press). The pH of plant vacuoles has never been artificially raised, nor have any industrially useful traits been obtained, and its association with flower color is unknown.

It is known that the Na*-H* antiporter gene, with a molecular weight of about 70,000, has been cloned from Arabidopsis, and a yeast into which this gene was introduced has acquired salt tolerance (Gaxiola et al., Proc. Natl. Acad. Sci. USA 96:1480-1485 (1999)), but it is not known how this antiporter regulates the pH of vacuoles in plant cells or how it is associated with

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flower color.

On the other hand, in petunias, seven loci are known to be involved in the pH regulation of petal vacuoles, and it has been proposed that the pH of petal vacuoles increases when one of them turns homozygously recessive (van Houwelingen et al., Plant J. 13:39 (1998); Mol et al., Trends Plant Sci. 3:212 (1998)). One of them, Ph6, has already been cloned and was found to be a kind of transcription regulating factor (Chuck et al., Plant Cell 5:371 (1993)), but the actual biochemical mechanism involved in the pH regulation of vacuoles is unknown.

In morning glory (Ipomea nil), the analysis of mutants revealed that a number of loci are associated with the color and shape of leaves and flowers and that 19 of them are highly mutable (Iida et al., Shokubutsu Saibo Koqaku Series (Plant Cell Engineering Series) 5 (1996) pp. 132, Shujunsha; Iida et al., Annal. New York Acad. Sci. (1999) pp. 870). Among them, the one locus defined by the recessive mutation that results in purple flowers instead of blue flowers is termed the Purple locus (T. Hagiwara, The genetics of flower colours in Phrarbitis nil. J. Coll. Agr. Imp. Univ. Tokyo 51:241-262 (1931); Y. Imai, Analysis of flower colour in Pharbitis nil. J. Genet. 24:203-224 (1931)), and one allele of mutable mutation that results in flowers that produce blue sectors in purple petals was termed purple-mutable (pr-m) (Imai, J. Coll. Agric. Imp. Univ. Tokyo 12:479 (1934)). The gene derived from the Purple locus is termed Purple gene.

The blue portion is believed to be derived from somatic reverse mutation from the recessive purple, and germ cell revertants can also be separated. An allele produced from the reverse mutation of these revertants are termed herein Purple-revertant (Pr-r). Such a classical method of genetic analysis had been performed on this Purple gene, but the identity of the Purple gene and its association etc. with the pH regulation of petal

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vacuoles were totally unknown.

It is believed that if the pH of vacuoles could be modified, for example if the pH of vacuoles could be raised, flower color could be turned blue.

Representative plant species that lack blue colors include roses, chrysanthemums, carnations, gerberas and the like, which are very important cut flowers. Though the importance of modifying pH of vacuoles has been recognized, the identities of proteins that regulate the pH of petal vacuoles are unknown and therefore the isolation of genes encoding them has been in great demand.

Disclosure of the Invention

The present invention provides a gene of a protein that regulates the pH of vacuoles in plant cells, preferably a gene of a protein that transports protons in vacuoles, more preferably a Na^+-H^+ antiporter gene. By introducing the gene of the present invention into a plant and allowing it to be expressed, flower color can be controlled and, preferably, can be turned blue.

Thus, the present invention provides a gene encoding a protein that regulates the pH of vacuoles. This gene is, preferably, a gene encoding a Na*-H* antiporter, for example a gene encoding a protein that has the amino acid sequence as set forth in SEQ ID NO: 2, or a gene encoding a protein that has an amino acid sequence modified by the addition or deletion of one or a plurality of amino acids and/or substitution with other amino acids in the amino acid sequence as set forth in SEO ID NO: 2 and that has an activity of regulating the pH of vacuoles; a gene encoding a protein that has an amino acid sequence having a identity of 20% or more with the amino acid sequence as set forth in SEQ ID NO: 2 and that has an activity of regulating the pH of vacuoles; or, a gene that hybridizes to part or all of a nucleic acid having a nucleotide sequence encoding the amino acid sequence as set forth in

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SEQ ID NO: 2 under a stringent condition, and that encodes a protein having an activity of regulating the pH of vacuoles.

The present invention also provides a vector comprising the above gene.

The present invention also provides a host cell transformed with the above vector.

The present invention also provides a protein encoded by the above gene.

The present invention further provides a method of producing a protein that has an activity of regulating the pH of vacuoles, said method comprising culturing or growing the above host cell and then harvesting said protein from said host cell .

The present invention also provides a plant in which said gene or said vector has been introduced or an progeny thereof having the same property as said plant, or a tissue thereof.

The present invention also provides a cut flower of the above plant or an progeny thereof.

The present invention further provides a method of regulating the pH of vacuoles comprising introducing the above gene or the above vector into a plant or plant cells and then allowing it to be expressed.

The present invention further provides a method of controlling the flower color of plants comprising introducing the above gene or the above vector into a plant or plant cells and then allowing said gene to be expressed.

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Brief Explanation of the Drawings

Fig. 1 is a drawing showing the structure of plasmid $\ensuremath{\mathsf{pSPB607}}.$

Fig. 2 is a drawing showing the structure of plasmid $\ensuremath{\mathsf{pSPB608}}\xspace.$

Fig. 3 is a drawing showing the structure of plasmid pINA145.

Fig. 4 is a drawing showing the structure of plasmid pINA147.

Best Mode for Carrying Out the Invention

The color of the petal of morning glory is blue when the locus Purple is dominant, and the blue petal turns purple when it is homozygously recessive. It is clear that the locus is associated with flower color but the mechanism thereof is unknown.

First, the chemical analysis of the pigments in the petal of the pr-m mutant and a revertant thereof detected no difference in the composition of the pigments. The change in flower color of the blue-colored morning glory from the reddish purple buds to the blue flowers accompanied by flowering is believed, as mentioned above, to be caused by pH changes in the vacuole of petal cells.

In the pr-m mutant, flowering is not associated with a color change to blue, and the pH of vacuoles of petal cells of flowers that bloomed was lower in the pr-m mutant than in Pr-r. Thus, the Purple gene is considered to be a gene that regulates the pH of vacuoles of petal cells during flowering and thereby controls flower color. Accordingly, using a pr-m mutant, and a revertant thereof, by the transposon display method, fragments of genomic DNA containing the Purple gene sequence specifically present in pr-m were identified and then the Purple gene was identified. Surprisingly, the Purple gene thus obtained had a homology with the Na*-H* antiporter from Arabidopsis etc., and, in the pr-m mutation, a transposon had been inserted in the 5'-untranslated region the Purple gene.

As the gene of the present invention, there can be mentioned, for example, one that encodes the amino acid sequence as set forth in SEQ ID NO: 2. It is known, however, that proteins having an amino acid sequence modified by the addition or deletion of one or a plurality of amino acids and/or substitution with other

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amino acids also retain an activity equal to that of the original protein. Thus in accordance with the present invention, a protein that has an amino acid sequence modified by the addition or deletion of one or a plurality of amino acids and/or substitution with other amino acids in the amino acid sequence as set forth in SEQ ID NO: 2, and a gene encoding said protein, are encompassed in the present invention as long as the protein is a protein that has an activity of regulating the pH of vacuoles.

The present invention also relates to a gene that hybridizes to the nucleotide sequence as set forth in SEQ ID NO: 1, a nucleotide sequence encoding the amino acid sequence as set forth in SEQ ID NO: 2, or a nucleotide sequence encoding part of these nucleotide sequences at a stringent condition, for example at 5 x SSC and 50°C, and that encodes a protein having an activity of regulating the pH of vacuoles. As used herein, a suitable hybridization temperature varies with the nucleotide sequence and the length of the nucleotide sequence, and when, for example, a DNA fragment comprising 18 bases encoding 6 amino acids is used as a probe, a temperature of 50°C or lower is preferred.

Genes selected, based on such hybridization, include those obtained from nature, for example from plants such as petunia and torenia, but a gene derived from sources other than plants may be used. Genes selected based on hybridization may be cDNA or genomic DNA.

The Na'-H' antiporter genes form a superfamily (Debrov et al., FEBS Lett. 424:1 (1998)), and have an amino acid homology of 20% or more (Orlowski et al., J. Biol. Chem. 272:22373 (1997)).

Thus, the present invention relates to a gene encoding a protein that has an amino acid sequence with a homology of about 20% or more, preferably 50% or more, for example 60% or 70% or more, and that has an activity of regulating the pH of vacuoles.

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A gene having an intact nucleotide sequence is obtained, as specifically illustrated in Examples, by, for example, screening cDNA libraries. DNA encoding a protein having a modified amino acid sequence can be synthesized by commonly used site-directed mutagenesis or the PCR method based on DNA having an intact nucleotide sequence. For example, a DNA fragment that is to be modified may be obtained by restriction enzyme treatment of the intact cDNA or genomic DNA, which is used as a template in the site-directed mutagenesis, or by the PCR method using primers in which desired mutation has been introduced to obtain a DNA fragment in which the desired modification has been introduced. Thereafter, the mutated DNA fragment may be ligated to a DNA fragment encoding another portion of the enzyme of interest.

Alternatively, in order to obtain DNA encoding a protein comprising a shortened amino acid sequence, an amino acid sequence longer than the amino acid sequence of interest, for example, DNA encoding the full-length amino acid sequence, may be cleaved with a desired restriction enzyme, and when the resultant DNA fragment was found not to encode the entire amino acid sequence of interest, a DNA fragment comprising the sequence of the lacking portion may be synthesized and ligated thereto.

The present invention is not limited to a gene encoding a protein that has an activity of regulating the pH of vacuoles derived from morning glory, but the sources may be plants, animals, or microorganisms, and all they need is to have a topology that pumps protons out of the vacuole.

By expressing the obtained gene using a gene expression system in Escherichia coli or yeast and determining the activity, it can be confirmed that the gene obtained encodes a protein that has an activity of regulating the pH of vacuoles. Furthermore, by expressing said gene, a protein, the gene product, having an activity of regulating the pH of vacuoles can be

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obtained. Alternatively, a protein can also be obtained that has an activity of regulating the pH of vacuoles using an antibody against the amino acid sequence as set forth in SEQ ID NO: 2, and a protein that has an activity of regulating the pH of vacuoles derived from other organisms can be cloned using an antibody.

Thus, the present invention also relates to a recombinant vector comprising the above-mentioned gene, specifically an expression vector, and a host cell transformed with said vector. As a host, there can be used a prokaryotic or eukaryotic organism. As a prokaryotic organism, for example, there can be used such a common host as a bacterium belonging to the genus Escherichia such as Escherichia coli, a bacterium belonging to the genus Bacillus such as Bacillus subtilis, and the like. As a eukaryotic host, there can be used a lower eukaryotic organism, for example an eukaryotic microorganism such as a fungus, a yeast or a mold.

As yeast, there can be mentioned a microorganism belonging to the genus Saccharomyces such as Saccharomyces cerevisiae, and as a mold, there can be mentioned a microorganism belonging to the genus Aspergillus such as Aspergillus oryzae and Aspergillus niger, and a microorganism belonging to the genus Penicillium. Furthermore, animal cells or plant cells can be used: as animal cells, there can be used cell lines derived from mouse, hamster, monkey, human and the like. Insect cells such as silkworm cells or adult silkworms per se can also be used as hosts.

The vectors of the present invention may contain expression regulatory regions such as a promoter, a terminator, an origin of replication, and the like, depending on the type of the host into which said vector is to be introduced. As promoters for bacterial expression vectors, there can be used commonly used promoters such as trc promoter, tac promoter, lac

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promoter, and the like; as promoters for yeasts, there can be used the glyceraldehyde-3-phosphate dehydrogenase promoter, PHO5 promoter, and the like; and as mold promoters, there can be used amylase promoter, trpC promoter, and the like.

As promoters for animal cell hosts, there can be used viral promoters such as SV40 early promoter, SV40 late promoter, and the like. The construction of expression vectors may be performed according to conventional methods using restriction enzymes, ligase, etc. The transformation of host cells can also be performed according to conventional methods.

Host cells transformed with the above expression vectors may be cultured, cultivated or bred, and from the culture the desired protein can be recovered and purified according to conventional methods such as filtration, centrifugation, cell disruption, gel filtration chromatography, ion exchange chromatography, and the like.

The present invention also relates to a plant or its progenies or tissues thereof of which hue of color has been controlled by introducing a gene encoding a protein that has an activity of regulating the pH of the vacuoles, specifically a Na⁺-H⁺ antiporter gene. They may be cut flowers in shape. Using a gene encoding a protein that has an activity of regulating the pH of vacuoles obtained by the present invention, the pumping of proton into the cytoplasm from the vacuole and the pumping of sodium ion into the vacuole can be performed, so that anthocyanins accumulated in the vacuole can be turned blue and, as a result, the flower color can be turned blue.

It is also possible to lower the pH of vacuoles by suppressing the expression of the gene of the present invention. With the state-of-the-art technology, it is possible to introduce a gene into plants, and allow the gene to be expressed in a constitutive or tissue-specific

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manner, and also to suppress the expression of the gene of interest by the antisense method or the co-suppression method.

Examples of plants that can be transformed include, but not limited to, roses, chrysanthemums, carnations, snapdragons, cyclamens, orchids, lisianthus, freesias, gerberas, gladioluses, gypsophilas, kalanchoes, lilies, pelargoniumas, geraniums, petunias, torenias, tulips, rice, barley, whieat, rapeseeds, potatoes, tomatoes, poplars, bananas, eucalyptuses, sweet potatoes, soy beans, alfalfas, lupins, corns, and the like.

Examples

The present invention will now be explained in further details with reference to the following Examples. Molecular biological techniques used were performed according to Molecular Cloning (Sambrook et al., 1989), unless otherwise specified.

Example 1. Obtaining a germ cell revertant

Obtaining a germ cell revertant has already been reported (Iida et al., Shokubutsu Saibo Kogaku Series (Plant Cell Engineering Series) 5 (1996) pp. 132, Shujunsha; Iida et al., Annal. New York Acad. Sci. (1999) pp. 870; Inagaki et al., Plant Cell, 6:375 (1994); Inagaki et al., Theor. Appl. Genet. 92:499 (1996)).

Morning glory having the genotype (Pr-r/pr-m) (Iida et al., pp. 870; Inagaki et al., Plant Cell, 6:375 (1994); Inagaki et al., Theor. Appl. Genet. 92:499 (1996)) was subjected to self-fertilization and the seeds of the progeny were planted. The flowers of the self-fertilized progeny were observed to select individuals that bloom with blue flowers by back mutation. Furthermore, in this self-fertilized progeny of the germ cell revertant, it was proved whether it is homozygous or heterozygous based on whether or not isolates that bloom with purple flowers can be obtained. Those having the genotype (Pr-r/Pr-r) and (pr-m/pr-m) were selected.

Example 2. Anthocyanins in the petals of revertants

Anthocyanins contained in morning glory are mainly heavenly blue anthocyanin and several other anthocyanins (Lu et al., Phytochemistry 31:659 (1992)). When the open petals of the Pr-r/Pr-r strain and the pr-m/pr-m strain obtained in Example 1 were similarly analyzed, the anthocyanins contained in both of them were almost identical.

A cellophane tape was stuck to the front side of a petal and then peeled off to recover one layer of epithelium, from which the cell liquid was scraped with a scalpel etc., which was then centrifuged to obtain juice. The pH of the juice was measured using the Horiba B212 pH meter (Horiba Seisakusho). pH of the petal epithelium of the Pr-r/Pr-r strain was about 7.1 whereas that of the pr-m/pr-m strain was about 6.5. This result indicates that the change in flower color by mutation of purple was not due to the structure of anthocyanins but to the change of vacuolar pH.

Example 3. Isolation of a genome fragment specifically present in pr-m

For the isolation of a gene, the transposon display method (Frey et al., Plant J. 13:717 (1998); Van den Broeck et al., Plant J. 13:121 (1998)) or a similar method (Dosho et al., Shokubutsu Saibo Kogaku Series (Plant Cell Engineering Series) 7 (1997) pp. 144, Shujunsha) was used to search for DNA bands that were present in the pr-m/pr-m strain and the Pr-w/pr-m strain but not in the Pr-r/Pr-r strain or in the wild strain. Since Tpnl-related transposon is thought to be mainly associated with mutability in morning glory, special note was given to the Tpnl-related transposon.

Specifically, chromosomal DNA was extracted from the pr-m/pr-m strain, and 125 ng of it was digested with MseI in 20 μ l. To the digested DNA was added 80 pmole of MseI adaptor (obtained by annealing 5'-GACGATGAGTCCTGAG-3' (SEQ ID NO: 3) and 5'-TACTCAGGACTCAT-3' (SEQ ID NO: 4))

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in 25 μ l at 20°C for 2 hours. After keeping it at 75°C for 10 minutes, it was stored at -20°C. After diluting this ten-fold, 2 μ l was used as a template, which was PCR-amplified using 4.8 pmole of TIR primer (5'-TGTGCATTTTCTTGTAGTG-3' (SEQ ID NO: 5), this includes the inverted terminal repeat of the transposon Tpn1) and 4.8 pmole of MseI primer (5'-GATGAGTCCTGAGTAA-3') (SEQ ID NO: 6) in 20 μ l.

PCR was performed with Taq polymerase (Takara Shuzo) for 20 cycles with one cycle comprising 94°C for 0.5 minute, 56°C for 1 minute, and 72°C for 1 minute, and the volume was diluted ten-fold. Two µl of it was used as a template in a PCR using 4.8 pmole of TIR+N primer (5'-TGTGCATTTTCTTGTAGN-3' (SEQ ID NO: 7) N=A, C, G or T. Four different species were synthesized instead of a mixture) and 4.8 pmole of MseI+N primer (5'-GATGAGTCCTGAGTAAN-3' (SEQ ID NO: 8) N=A, C, G or T. Four different species were synthesized instead of a mixture. The 5'-end was labeled with fluorescein (using Amersham Pharmacia Biotek, Vistra fluorescence 5'-oligo labeling kit)) in 20 µl.

Reactions were performed for combinations of primers to a total of 16 reactions. PCR was performed for 13 cycles with one cycle comprising 94°C for 0.5 minute, 65°C (with a decrement of 0.7°C for each cycle) for 1 minute, and 72°C for 1 minute, and further for 13 cycles with one cycle comprising 94°C for 0.5 minute, 56°C for 1 minute, and 72°C for 1 minute. A similar procedure was performed for chromosomal DNA obtained from the Pr-r/Pr-r strain, subjected to electrophoresis using a sequence gel of the DNA Sequencer 377 (PE Biosystems Japan), and the bands were detected using FMBIOII (Takara Shuzo).

When bands derived from the Pr-r/Pr-r strain and the pr-m/pr-m strain were compared, an about 130 bp DNA fragment was specifically expressed in the strain having

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pr-m. The 130 bp DNA fragment was recovered, and amplified by PCR (for 30 cycles with one cycle comprising 94°C for 0.5 minute, 56°C for 1 minute, and 72°C for 1 minute) using 20 pmole TIR primer and 20 pmole MseI primer, which was then subcloned into the pGEM-T vector (Promega Corporation), and then the nucleotide sequence was determined. The sequence was

5'-TGAGCATTTTCTTGTAGTG CTGAGATTTTCCTCCATTTGTCTGAAGCTCTTCAACCC
TACCCCCAGATCTCACCTTTCAAG GTCCAATCTTTATCATTCATCT TTACTCAGGACTCATCGTC-3'

(SEQ ID NO: 9) (the single-underlined portion corresponds to a used primer, the double-underlined portion corresponds to an exon, and the rest corresponds to an intron). After the sequence as set forth in SEQ ID NO: 9 was used as a probe in Northern analysis, a transcription product of about 2.3 kb was found in the bud of morning glory having Pr-r, but a corresponding transcription product was not found in the pr-m/pr-m strain. Thus, it can be seen that this 2.3 kb transcription product corresponds to the Purple gene.

Example 4. Isolation of cDNA

About 6 million clones of a cDNA library (Inagaki et al., Plant Cell 6:375 (1994)) derived from the wild strain morning glory (Pr-w/Pr-w) were screened using the 130 bp DNA fragment as a probe, with a result that two positive clones were obtained. One of these clones had a 2237 bp cDNA, among which a 1626 bp-long open reading frame was observed (SEQ ID NO: 1). The predicted amino acid sequence had an identity of 29.3% and 73.4% with the Na'-H' antiporter of yeast and Arabidopsis, respectively (Nhx1 and AtNhx1, respectively, Gaxiola et al., Proc. Natl. Acad. Sci. USA 96:1480-1485 (1999)).

The result revealed that the Purple gene of morning glory encodes a Na'-H' antiporter. Incidentally, although the Na'-H' antiporter obtained from Arabidopsis is attracting attention as a protein that gives salt resistance to yeast, this is the first time that an association of the Na'-H' antiporter with flower color

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was observed.

Example 5. Complementation experiment of yeast Na*-H* antiporter

The predicted amino acid sequence encoded by the Purple gene of morning glory has a homology with those of the Na^{*}-H^{*} antiporters of yeast and Arabidopsis. Thus, in order to confirm whether the Purple gene product of morning glory can function as a Na^{*}-H^{*} antiporter protein, a complementation experiment was performed using a yeast Na^{*}-H^{*} antiporter mutant.

First, the following two DNA fragments were synthesized:

CBSC1-Linker (22 mer) 5'-CGA TAG ATC TGG GGG TCG ACA T-3' (SEQ ID NO: 12)

CSBD2-Linker (22 mer) 5'-CGA TGT CGA CCC CCA GAT CTA T-3' (SEQ ID NO: 13)

From these two fragments, a linker having

restriction enzyme sites ClaI-BglII-SalI-ClaI is formed. A plasmid pINA145 (Fig. 3) was constructed by inserting the above linker according to a standard method into the ClaI site of the pYES2 vector (Invitrogen Corporation) so that the BqlII site is located at the URA3 gene side. A plasmid pINA147 (Fig. 4) was constructed by ligating a 2 kb DNA fragment obtained by digesting plasmid pJJ250 (Jones and Prakash, Yeast 6:363-366 (1990)) with BamHI and SalI to plasmid pINA145 digested with BglII and SalI. Plasmid pIAN151 was constructed by ligating Purple cDNA thereto under the control of the GAL 1 promoter of plasmid pINA147. pINA147 and pIAN151 were transformed respectively to the yeast R101 strain which is a mutant strain of the $\mathrm{Na}^{\scriptscriptstyle{+}}\mathrm{-H}^{\scriptscriptstyle{+}}$ antiporter. Due to the mutation of the Na+-H+ antiporter, the yeast R101 strain cannot grow on a 400 mM NaCl-added APG medium (Nass et al., J. Biol. Chem. 272:26145 (1997); Gaxiola et al., 96:1480-1485 (1999)). The pINA147-transformed R101 strain could not grow either, and only the pIAN151-transformed R101 strain

could grow on the 400 mM NaCl-added APG medium.

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result has shown that the gene product of the morning glory Purple gene has the Na*-H* antiporter function.

Example 6. Construction of an expression vector in plants

With 10 ng of morning glory Purple cDNA as template, PCR was performed using synthetic primers PR-5 (5'-GGGATCCAACAAAATGGCTGTCGGG-3') (SEQ ID NO: 10) and PR-3 (5'-GGGTCGACTAAGCATCAAAACTAGAGCC-3') (SEQ ID NO: 11). The polymerase used was Taq polymerase (Toyoboseki), and the reaction was performed, after reaction at 95°C for 45 seconds, for 25 cycles with one cycle comprising 95°C for 45 seconds, 50°C for 45 seconds, and 72°C for 45 seconds, and then further reacted at 72°C for 10 minutes. An about 1.6 kb DNA fragment obtained was ligated to pCR2.1-Topo (Clontech) to make pCR-purple. It was confirmed that there were no errors due to PCR in the nucleotide sequence of Purple cDNA on this plasmid.

pBE2113-GUS (Mitsuhara et al., Plant Cell Physiol. 37:49 (1996)) was digested with SacI and blunt-ended. Then a XhoI linker (Toyoboseki) was inserted thereto, and the plasmid obtained was termed pBE2113-GUSX. This was digested with EcoRI and HindIII to obtain an about 2.7 kb DNA fragment, which was ligated to the HindIII and EcoRI digest of pBinPLUS, and the plasmid obtained was termed pBEXP.

On the other hand, an about 1.2 kb DNA fragment obtained by digesting pCGP484 (Kohyo (National Publication of Translated Version) No. 8-511683) with HindIII and XbaI, an about 1.6 kb DNA fragment obtained by digesting pCR-purple with XbaI and SalI, and an about 13 kb DNA fragment obtained by digesting pEEXP with HindIII and XhoI were ligated to obtain pSPB607 (Fig. 1). This plasmid is a binary vector for use in the Agrobacterium-mediated transformation of plants, and on this plasmid Purple cDNA is under the control of a chalcone synthase promoter derived from snapdragon and a nopaline synthase terminator derived from Agrobacterium.

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An about 0.8 kb DNA fragment obtained by digesting pCGP669 (Kohyo (National Publication of Translated Version) No. 8-511683) with HindIII and BamHI, an about 1.6 kb DNA fragment obtained by digesting pCR-purple with BamHI and SalI, and an about 13 kb DNA fragment obtained by digesting pBEXP with HindIII and XhoI were ligated to obtain pSPB608 (Fig. 2). This plasmid is a binary vector for use in the Agrobacterium-mediated transformation of plants, and on this plasmid Purple cDNA is under the control of a chalcone synthase promoter derived from petunia and a nopaline synthase terminator derived from Agrobacterium.

By transforming plants using the expression vectors thus obtained, the pH of vacuoles can be regulated and thereby flower color can be controlled. Example 7. Isolation of a homologs of the Purple gene

cDNA libraries derived from the petals of petunia (Petunia hybrida cv. Old Glory Blue), Nierembergia (Nierembergia hybrida cv. NB17), and Torenia (Torenia hybrida cv. Summerwave Blue) were each constructed using the cDNA synthesis kit (Stratagene, USA). The method of construction was as recommended by the manufacturer. About 200,000 clones each were screened according to a standard method. For washing the membrane, an aqueous solution of 5 \times SSC and 0.1% SDS was used and the incubation was performed three times at 50°C for 10 minutes. Among the positive clones obtained, the nucleotide sequence of the longest clone was determined for each clone. The nucleotide sequence of the clone of Petunia and the corresponding amino acid sequence are shown in SEQ ID NO: 14 and 15, the nucleotide sequence of the clone of Nierembergia and the corresponding amino acid sequence are shown in SEQ ID NO: 16 and 17, and the nucleotide sequence of the clone of Torenia and the corresponding amino acid sequence are shown in SEQ ID NO: 18 and 19. Homologs of the Purple gene of Petunia, Nierembergia, and Torenia had an identity on the amino

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acid level of 75%, 76%, and 71%, respectively, with the morning glory Purple gene.

Since the amino acid sequence of the Na*-H* antiporter encoded by the morning glory Purple gene and that of the Na*-H* antiporter encoded by Arabidopsis AtNhx 1 are about 73% identical, the homologs of the Purple gene of Petunia, Nierembergia, and Torenia obtained are judged to encode the Na*-H* antiporter. Example 8. Isolation of the clone of morning glory

10 Purple chromosome

After chromosomal DNAs of a mutant morning glory (pr-m/pr-m) and a revertant morning glory (Pr-r/Pr-r) were cleaved with BglII, they were electrophoresed on a 0.8% agarose gel, and were subjected to genomic Southern analysis with cDNA of morning glory Purple as a probe. As a result, an about 7.5 kb band that was not present in the mutant morning glory was detected in the revertant morning glory.

After 50 µg of chromosomal DNA of the wild type morning glory (Pr-w/Pr-w, the KKZSK2 strain) was digested with BglII, it was electrophoresed on a 0.8% agarose gel. An about 7-9 kb fragmently was recovered, from which DNA was extracted using the GENECLEAN III KIT (B10101). This DNA was ligated to the \(\lambda\) Zap express vector (Stratagene, USA), which was screened with cDNA of morning glory Purple as a probe. The determination of nucleotide sequences of positive clones obtained revealed that, on this about 7.5 kb DNA fragment, there was a region from about 6.3 kb upstream of the Purple promoter to midway in exon 3. For this sequence, a sequence up to the initiation codon of the Purple gene is shown in SEQ ID NO: 20.

It has been demonstrated that the expression of the Purple gene is strongly induced only at about 24 hours before the flowering of morning glory, and that the expression of the Purple gene is suppressed by insertion

of a transposon into the 5'-untranslated region. From this, it is clear that the promoter region of the Purple gene obtained contains a factor needed for the expression of the Purple gene in a developmental stage-specific and organ-specific manner in the petals of morning glory. By placing the gene of interest downstream of this promoter region, the expression of the gene of interest can be regulated in a developmental stage-specific and organ-specific manner.

10 Industrial Applicability

The gene obtained in the present invention was found, for the first time, to be involved in controlling the pH of vacuoles and flower color. By expressing the gene of the present invention on the flower petals, the pH of vacuoles can be increased and thereby the flower color can be turned blue. Furthermore, by suppressing the expression of the gene of the present invention, the pH of vacuoles can be lowered and thereby flower color can be turned red. As the gene encoding a protein that regulates the pH of vacuoles, there can be used not only those derived from morning glory obtained in the present invention but also similar genes derived from other organisms.

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CLAIMS

- 1. A gene encoding a protein that has an activity of regulating the pH of vacuoles in plant cells.
- 2. A gene encoding a protein that has the amino acid sequence as set forth in SEQ ID NO: 2 and that has an activity of regulating the pH of vacuoles in plant cells.
- 3. A gene encoding a protein that has an amino acid sequence modified by the addition or deletion of one or a plurality of amino acids and/or substitution with other amino acids in the amino acid sequence as set forth in SEQ ID NO: 2 and that has an activity of regulating the pH of vacuoles.
- 4. The gene according to claim 1 encoding a protein that has an amino acid sequence having a identity of 20% or more with the amino acid sequence as set forth in SEQ ID NO: 2 and that has an activity of regulating the pH of vacuoles.
- 5. The gene according to claim 1 encoding a protein that has an amino acid sequence having a identity of 70% or more with the amino acid sequence as set forth in SEQ ID NO: 2 and that has an activity of regulating the pH of vacuoles.
- 6. The gene according to claim 1 that hybridizes to a part or all of a nucleic acid having a nucleotide sequence encoding the amino acid sequence as set forth in SEQ ID NO: 2 under a stringent condition, and that encodes a protein having an activity of regulating the pH of vacuoles.
- 30 7. A vector comprising the gene according to any one of the claims 1 to 6.
 - 8. A host cell transformed with the vector according to claim 7.
- 9. A protein encoded by the gene according to any $\,$ one of the claims 1 to 6.
 - $10.\,\,$ A method of producing a protein that has an activity of regulating the pH of vacuoles, said method

comprising culturing or growing the host cell according to claim 8 and then harvesting said protein from said host cell.

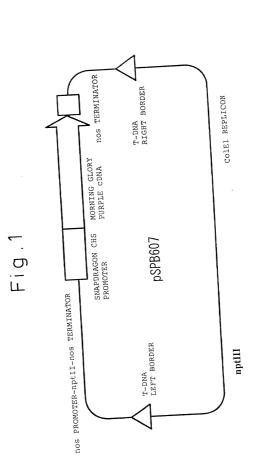
- 11. A plant in which the gene according to any one of the claims 1 to 6 or the vector according to claim 7 has been introduced or an progeny thereof having the same property as said plant, or a tissue thereof.
- 12. A cut flower of the plant according to claim 11 or an progeny thereof having the same property as said plant.
- 13. A method of regulating the pH of vacuoles comprising introducing the gene according to any one of the claims 1 to 6 or the vector according to claim 7 into a plant or plant cells and then allowing said gene to be expressed.
- 14. A method of controlling the flower color of plants comprising introducing the gene according to any one of the claims 1 to 6 or the vector according to claim 7 into a plant or plant cells and then allowing said gene to be expressed.

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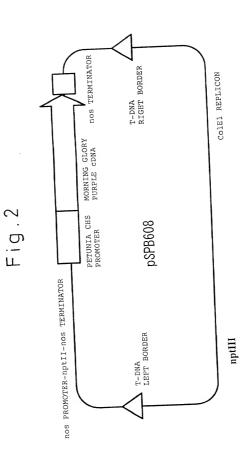


Fig.3

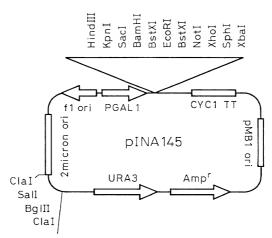


Fig.4

f1 ori PGAL1 CYC1 TT pMB1 ori

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Declaration and Power of Attorney For Patent Application

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日本語宣言書

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I hereby claim the benefit under Title 35, United States Code, Section 119(e) of any United States provisional application(s) listed below.

(Application No.) (Filing Date) (出類音号) (出類目)

i hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s), or 356(c) of any PCT International application designating the United States, listed below and, insolar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code Section 112, I acknowledge the duty to disclose Information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.58 which became available between the filling date of the prior application and the national or PCT International filling date of application.

(Status: Patented, Pending, Abandoned) (现況:特許許可簽、係属中、故棄簽)

> (Status: Patented, Pending, Abandoned) (現況: 特許許可濟、保属中、数案済)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful faise statements and the like so made are punishable by fine or impaisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilfullir faise statements may jeopardize the validity of the application or any patent issued thereon.

PTO/SB/106 (6-96)
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Japanese Language Declaration (日本語宣言書)

委任状: 私は下記の発明者として、本出額に関する一切の 子続きを米特許商標局に対して遂行する弁理士または代理人 として、下記の者を指名いたします。(弁護士、または代理 人の氏名及び登録番号を明記のこと)

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| 国 | 籍 | | Citizenship | |
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| 第六 | 共同発明者 | 日付 | Sixth inventor's signature | Date |
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| 国 | 籍 | | Citizenship | |
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Page 4 of 4

SEQUENCE LISTING

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| Leu | Phe | Asn | Ala | Ile | Gln | Ser | Phe | Asp | Met | Thr | Ser | Phe | Asp | Pro | Lys | |
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| Ųggt | atg | gat | gcc | ttg | gat | atc | gag | aaa | tgg | aaa | ttt | gtg | aaa | aat | agt | 1307 |
| Gly | Met | Asp | Ala | Leu | Asp | Ile | Glu | Lys | Trp | Lys | Phe | Val | Lys | Asn | Ser | |
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| Val | ${\tt Gly}$ | Arg | Ala | Ala | Phe | Val | Phe | Pro | Leu | Ser | Phe | Leu | Ser | Asn | Leu | |
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| Ala | Lys | Lys | Asn | Ser | Ser | Asp | Lys | Ile | Ser | Phe | Arg | Gln | Gln | Ile | Ile | |
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| tat | aat | aag | ttt | aca | acc | tcg | ggg | cat | acg | tca | ttg | cac | gag | aac | gca | 1547 |
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| ata | atg | att | aca | agt | act | gtt | acg | gtt | gtt | ctg | ttc | agc | aca | gtt | gta | 1595 |
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| aaaa | aaaa | aa | | | | | | | | | | | | | | 2237 |
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| <212 | ?> | PF | Y.T. | | | | | | | | | | | | | |

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<223> Amino acid sequence of protein regulating the pH of vacuoles

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| Ser | Asp | His | Ala | Ser | Val | Val | Ser | Met | Asn | Leu | Phe | Val | Ala | Leu | Leu |
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| Cys | Ala | Cys | Ile | Val | Leu | Gly | His | Leu | Leu | Glu | Glu | Asn | Arg | Trp | Val |
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| Glu | Asp | Leu | Phe | Phe | Ile | Tyr | Leu | Leu | Pro | Pro | Ile | Ile | Phe | Asn | Ala |
| | | | | 85 | | | | | 90 | | | | | 95 | |
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| WMet | Leu | Phe | Gly | Ala | Ile | Gly | Thr | Leu | Ile | Ser | Cys | Ser | Ile | Ile | Ser |
| p. de | | 115 | | | | | 120 | | | | | 125 | | | |
| UPhe | | Ala | Val | Lys | Ile | Phe | Lys | His | Leu | Asp | Ile | Asp | Phe | Leu | Asp |
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| Phe | Gly | Asp | Tyr | Leu | | Ile | Gly | Ala | Ile | | Ala | Ala | Thr | Asp | Ser |
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| Val | Cys | Thr | Leu | | Val | Leu | Ser | Gln | | Glu | Thr | Pro | Leu | Leu | Tyr |
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| Ser | Leu | val | | Gly | Glu | Gly | Val | | Asn | Asp | Ala | Thr | | Val | Val |
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| Leu | Phe | 195 | Ата | TTE | GIN | ser | | Asp | Met | Thr | ser | | Asp | Pro | Lys |
| Tlo | C1** | | 77.6 ~ | Db - | -1- | G3 | 200 | D1: | - | _ | _ | 205 | _ | _ | _ |
| iie | Gly 210 | Leu | HIS | Pne | TTE | | ASI | Pne | Leu | Tyr | | Pne | Leu | ser | Ser |
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| Thr | Phe | Leu | Glv | Val | Glv | Tle | G1 17 | T.017 | Len | Cvc | - ר מ | mar. | т1 с | Tle | T |
| 225 | 1110 | шей | GIY | vai | 230 | 116 | GIY | пеп | ьеи | 235 | Ala | TAT | тте | тте | Lys 240 |
| | Leu | Tvr | Phe | Glv | | Hie | Ser | Thr | Aen | | Glu | 17 a 1 | Δla | Ten | |
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| A Sport | Phe | GIY | 435 | мет | Thr | ьуs | Pro | | TIE | Asn | Leu | Leu | | Pro | Pro | His |
| 100 | | Cln | | Dwo | | C1 | 77.2 | 440 | | | m1 | m) | 445 | ~ 1 | _ | _ |
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| Ser | Thr | Ala | Leu | Gly | Val | Ala | Ala | Gly | Leu | Leu | Ser | Ala | Tyr | Ile | Ile |
| 225 | | | | | 230 | | | | | 235 | | | | | 240 |
| Lys | Lys | Leu | ${\tt Tyr}$ | Phe | Gly | Arg | His | Ser | Thr | Asp | Arg | Glu | Val | Ala | Ile |
| | | | | 245 | | | | | 250 | | | | | 255 | |
| Met | Ile | Leu | Met | Ala | Tyr | Leu | Ser | Tyr | Met | Leu | Ala | Glu | Leu | Phe | Tyr |
| | | | 260 | | | | | 265 | | | | | 270 | | |
| Leu | Ser | Ala | Ile | Leu | Thr | Val | Phe | Phe | Ser | Gly | Ile | Val | Met | Ser | His |
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| Tyr | Thr | Trp | His | Asn | Val | Thr | Glu | Ser | Ser | Arg | Val | Thr | Thr | Lys | His |
| | 290 | | | | | 295 | | | | | 200 | | | | |

| Thr | Phe | Ala | Thr | Leu | Ser | Phe | Ile | Ala | Glu | Ile | Phe | Ile | Phe | Leu | Tyr |
|-------|-----|-----|-----|-------|-----|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 305 | | | | | 310 | | | | | 315 | | | | | 320 |
| Val | Gly | Met | Asp | Ala | Leu | Asp | Ile | Glu | Lys | Trp | Lys | Phe | Val | Ser | Asp |
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| Ser | Pro | Gly | Ile | Ser | Val | Gln | Val | Ser | Ser | Ile | Leu | Leu | Gly | Leu | Val |
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| Leu | Val | Gly | Arg | Ala | Ala | Phe | Val | Phe | Pro | Leu | Ser | Phe | Leu | Ser | Asn |
| | | 355 | | | | | 360 | | | | | 365 | | | |
| Leu | | Lys | Lys | Thr | Pro | Glu | Ala | Lys | Ile | Ser | Phe | Asn | Gln | Gln | Val |
| | 370 | | | | | 375 | | | | | 380 | | | | |
| | | Trp | Trp | Ala | Gly | Leu | Met | Arg | Gly | Ala | Val | Ser | Met | Ala | Leu |
| 385 | | | | | 390 | | | | | 395 | | | | | 400 |
| (JAla | Tyr | Asn | Gln | Phe | Thr | Arg | Gly | Gly | His | Thr | Gln | Leu | Arg | Ala | Asn |
| (0 | | | | 405 | | | | | 410 | | | | | 415 | |
| []Ala | Ile | Met | Ile | Thr | Ser | Thr | Ile | Thr | Val | Val | Leu | Phe | Ser | Thr | Val |
| 944 | | | 420 | | | | | 425 | | | | | 430 | | |
| [UVal | Phe | | Leu | Met | Thr | Lys | Pro | Leu | Ile | Arg | Ile | Leu | Leu | Pro | Ser |
| \$1.J | | 435 | | | | | 440 | | | | | 445 | | | |
| (]His | | His | Leu | Ser | Arg | Met | Ile | Ser | Ser | Glu | Pro | Thr | Thr | Pro | Lys |
| ,E | 450 | | | | | 455 | | | | | 460 | | | | |
| Ser | Phe | Ile | Val | | | Leu | Asp | Ser | Thr | Gln | Asp | Ser | Glu | Ala | Asp |
| (1465 | _ | | | | 170 | | | | | 475 | | | | | 480 |
| Leu | GIu | Arg | His | Val | Pro | Arg | Pro | His | | Leu | Arg | Met | Leu | Leu | Ser |
| | _ | | | 485 | | | | | 490 | | | | | 495 | |
| rnr | Pro | Ser | | Thr | Val | His | Tyr | | Trp | Arg | Lys | Phe | | Asn | Ala |
| | | | 500 | | | | | 505 | | | | | 510 | | |
| Dho | Ma+ | | D | **- 1 | -1 | | | _ | | | | | | | |
| PHE | Met | 515 | Pro | Val | Phe | GLY | | Arg | Gly | Phe | Val | | Phe | Ala | Pro |
| C1** | 802 | | mh | D | D | ** - 7 | 520 | | _ | _ | | 525 | | | |
| GIY | 530 | PIO | THE | ASP | Pro | | GIY | GIY | Asn | Leu | | | | | |
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| <211 | | | 553 | | | | | | | | | | | | |
| <212 | > | DN | | | | | | | | | | | | | |

<213> Nierembergia hybrida

85

<223> Nucleotide sequence of DNA encoding for protein regulating the pH of vacuoles

| <40 | 0> | 1 | 6 | | | | | | | | | | | | | |
|----------|------|-----|------|------|------|-------|------|------|------|--------------|-----|------|-----|-------|--------|-----|
| aat | tatt | att | attt | ctct | cc a | actc | tcat | t tc | tcag | tttg | ttg | tgac | ttt | ttca | gagctt | 60 |
| gaa | gttc | agt | taat | tcat | tt t | ccaa | tata | t tg | attg | t ttt | cat | ttga | gcg | cgag | aggatt | 120 |
| tcg | tctt | ctc | aatc | tgct | tt d | caaat | cctt | t tt | gttt | gtga | tat | tcga | tat | tatt | cactca | 180 |
| gtt | tacc | tta | atat | ttcc | tc q | gcact | ttct | g aa | ttcg | agtg | ctt | tgaa | gtg | tgtt | ggattt | 240 |
| cga | aaag | cgg | aaga | aaat | tc a | agcaa | aaac | g ct | gttg | ctga | att | tgca | gca | gttt | gagttt | 300 |
| ttg | ctaa | ata | gcta | agat | ct ç | gattg | aatt | t tt | cact | ggtg | ctt | atag | gga | aatt | cgacgt | 360 |
| | | | | | | | | | | | | | | | tgaaat | |
| Ottg | aatg | taa | ggtt | gtca | ta ç | gcttt | gcca | c to | ggaa | atac | agt | cagt | gag | aaag | aaaaa | 480 |
| aac | tgtg | tag | tgtt | tttt | cc a | caag | tatt | t gg | tgaa | ttga | ggt | tctt | gaa | atg | gcg | 536 |
| 10 | | | | | | | | | | | | | | Met 1 | | |
| | | | | | | g ctg | | | | | | | | | | 584 |
| [UPhe | Asp | | Gly | Thr | Leu | Leu | Gly | Lys | Met | Asn | Asn | Leu | Thr | Thr | Ser | |
| [4] s | | 5 | | | | | 10 | | | | | 15 | | | | |
| gat | | | | | | | | | | | | | | | | 632 |
| - Asp | | Gln | Ser | Val | Val | Ser | Val | Asn | Leu | Phe | Val | Ala | Leu | Ile | Cys | |
| 2 04 | 20 | | | | | 25 | | | | | 30 | | | | | |
| gcg | tgt | att | gtg | atc | ggt | cat | tta | ttg | gag | gaa | aac | aga | tgg | atg | aat | 680 |
| P≜Ala | Cys | Ile | Val | Ile | Gly | His | Leu | Leu | Glu | Glu | Asn | Arg | Trp | Met | Asn | |
| 35 | | | | | 40 | | | | | 45 | | | | | 50 | |
| | | | | | | gtg | | | | | | | | | | 728 |
| Glu | Ser | Ile | Thr | | Leu | Val | Ile | Gly | | Cys | Thr | Gly | Val | Ile | Ile | |
| | | | | 55 | | | | | 60 | | | | | 65 | | |
| | | | | | | aag | | | | | | | | | | 776 |
| Leu | Leu | ше | | GIY | Gly | Lys | Asn | | His | Ile | Leu | Val | Phe | Ser | Glu | |
| | | | 70 | | | | | 75 | | | | | 80 | | | |
| | | | | | | ctt | | | | | | | | | | 824 |
| ASP | ьeu | rne | Pne | IIe | Tyr | Leu | Leu | Pro | Pro | Ile | Ile | Phe | Asn | Ala | Gly | |

95

| τ | tc | cag | gtg | aaa | aag | aaa | tca | ttc | ttc | cgc | aat | ttc | agt | act | atc | atg | 872 |
|--------|----|-----|-----|-------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| P | he | Gln | Val | Lys | Lys | Lys | Ser | Phe | Phe | Arg | Asn | Phe | Ser | Thr | Ile | Met | |
| | | 100 | | | | | 105 | | | | | 110 | | | | | |
| C | tc | ttt | ggg | gca | gtt | ggc | acc | ttg | ata | tcg | ttc | att | att | ata | tca | gcg | 920 |
| L | eu | Phe | Gly | Ala | Val | Gly | Thr | Leu | Ile | Ser | Phe | Ile | Ile | Ile | Ser | Ala | |
| 1 | 15 | | | | | 120 | | | | | 125 | | | | | 130 | |
| g | gt | gct | att | ggc | att | ttc | aag | aaa | atg | gat | att | gga | cac | ctt | gaa | att | 968 |
| G | ly | Ala | Ile | Gly | Ile | Phe | Lys | Lys | Met | Asp | Ile | Gly | His | Leu | Glu | Ile | |
| | | | | | 135 | | | | | 140 | | | | | 145 | | |
| g | ga | gat | tac | ctt | gca | att | gga | gca | atc | ttt | gct | gca | aca | gat | tct | gta | 1016 |
| G | ly | Asp | Tyr | Leu | Ala | Ile | Gly | Ala | Ile | Phe | Ala | Ala | Thr | Asp | Ser | Val | |
| | | | | 150 | | | | | 155 | | | | | 160 | | | |
| t | gc | acc | tta | caa | gtg | ctt | aat | cag | gaa | gaa | aca | ccg | tta | ttg | tac | agt | 1064 |
| C | ys | Thr | Leu | Gln | Val | Leu | Asn | Gln | Glu | Glu | Thr | Pro | Leu | Leu | Tyr | Ser | |
| ŭ O | | | 165 | | | | | 170 | | | | | 175 | | | | |
| į ci | ta | gtg | ttt | gga | gaa | ggt | gtt | gtg | aat | gat | gcc | aca | tct | gta | gtg | ctg | 1112 |
| Le | eu | Val | Phe | ${\tt Gly}$ | Glu | Gly | Val | Val | Asn | Asp | Ala | Thr | Ser | Val | Val | Leu | |
| Į. | | 180 | | | | | 185 | | | | | 190 | | | | | |
| 4tt | tc | aat | gct | gtc | cag | aac | ttt | gac | tta | tct | cat | atc | agc | aca | ggc | aaa | 1160 |
| Pł | he | Asn | Ala | Val | Gln | Asn | Phe | Asp | Leu | Ser | His | Ile | Ser | Thr | Gly | Lys | |
| -19 | 95 | | | | | 200 | | | | | 205 | | | | | 210 | |
| go | ct | ctg | caa | tta | att | gga | aac | ttt | cta | tac | ttg | ttt | gcc | tcg | agc | act | 1208 |
| JA. | la | Leu | Gln | Leu | Ile | Gly | Asn | Phe | Leu | Tyr | Leu | Phe | Ala | Ser | Ser | Thr | |
| zên. | | | | | 215 | | | | | 220 | | | | 225 | | | |
| tt | tc | cta | ggg | gtt | gct | gtt | ggc | cta | cta | agt | gcc | ttt | ata | att | aag | aaa | 1256 |
| Pł | ne | Leu | Gly | Val | Ala | Val | Gly | Leu | Leu | Ser | Ala | Phe | Ile | Ile | Lys | Lys | |
| | | | | 230 | | | | 235 | | | | | 240 | | | | |
| | | | | | | | | | gat | | | | | | | | 1304 |
| Le | ∍u | Tyr | Phe | Gly | Arg | His | Ser | Thr | Asp | Arg | Glu | Val | Ala | Ile | Met | Ile | |
| | | | 245 | | | | | 250 | | | | | 255 | | | | |
| ct | cc | atg | gcg | tac | cta | tca | tac | atg | ctt | gct | gaa | tta | ttc | tat | tta | agt | 1352 |
| Le | | | Ala | Tyr | Leu | Ser | Tyr | Met | Leu | Ala | Glu | Leu | Phe | Tyr | Leu | Ser | |
| | | 260 | | | | | 265 | | | | | 270 | | | | | |
| gç | ga | atc | ctc | act | gtg | ttt | ttc | tgt | ggg | atc | gtg | atg | tct | cac | tat | acc | 1400 |
| | | Ile | Leu | Thr | Val | | Phe | Cys | Gly | Ile | Val | Met | Ser | His | Tyr | Thr | |
| 27 | 75 | | | | | 280 | | | | | 285 | | | | | 290 | |

| -99 | cuc | uuc | gug | acc | yay | ayc | tCa | aya | 900 | act | acc | aag | cac | acg | ttt | 1448 |
|------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| Trp | His | Asn | Val | Thr | Glu | ser | Ser | Arg | Val | Thr | Thr | Lys | His | Thr | Phe | |
| | | | | 295 | | | | | 300 | | | | | 305 | | |
| gct | aca | tta | tca | ttt | att | gct | gaa | ata | ttc | ata | ttc | ctt | tat | gtt | ggt | 1496 |
| Ala | Thr | Leu | Ser | Phe | Ile | Ala | Glu | Ile | Phe | Ile | Phe | Leu | Tyr | Val | Gly | |
| | | | 310 | | | | | 315 | | | | | 320 | | | |
| atg | gat | gct | ttg | gac | att | gag | aag | tgg | aag | ttt | gta | agc | gac | agc | ccc | 1544 |
| Met | Asp | Ala | Leu | Asp | Ile | Glu | Lys | Trp | Lys | Phe | Val | Ser | Asp | Ser | Pro | |
| | | 325 | | | | | 330 | | | | | 335 | | | | |
| gga | aca | tca | att | aag | gtc | agc | tca | att | ctg | cta | ggt | ctt | gtt | ttg | gtt | 1592 |
| Gly | Thr | Ser | Ile | Lys | Val | Ser | Ser | Ile | Leu | Leu | Gly | Leu | Val | Leu | Val | |
| | 340 | | | | | 345 | | | | | 350 | | | | | |
| gga | agg | gga | gcc | ttt | gtt | ttc | ccc | ttg | tca | ttc | ttg | tcc | aac | ttg | acc | 1640 |
| Gly | Arg | Gly | Ala | Phe | Val | Phe | Pro | Leu | Ser | Phe | Leu | Ser | Asn | Leu | Thr | |
| 355 | | | | | 360 | | | | | 365 | | | | | 370 | |
| aag | aaa | aat | cct | gag | gac | aag | att | agc | ttt | aac | cag | cag | gtt | aca | ata | 1688 |
| Lys | Lys | Asn | Pro | Glu | Asp | Lys | Ile | Ser | Phe | Asn | Gln | Gln | Val | Thr | Ile | |
| Ų | | | | 375 | | | | | 380 | | | | | 385 | | |
| [∐] tgg | tgg | gct | ggg | ctt | atg | cga | ggt | gct | gtt | tct | atg | gcc | ctt | gct | tat | 1736 |
| Trp | Trp | Ala | Gly | Leu | Met | Arg | Gly | Ala | Val | Ser | Met | Ala | Leu | Ala | Tyr | |
| | | | 390 | | | | | 395 | | | | | 400 | | | |
| aat | | | | | | | | | | | | | | | | 1784 |
| Asn | Gln | | Thr | Arg | Gly | Gly | His | Thr | Gln | Leu | Arg | Ala | Asn | Ala | Ile | |
| | | 405 | | | | | 410 | | | | | 415 | | | | |
| | | | | | | | | gtc | | | | | | | | 1832 |
| Met | | Thr | Ser | | Ile | Thr | Val | Val | Leu | Phe | Ser | Thr | Val | Val | Phe | |
| | 420 | | | 425 | | | | | | | 430 | | | | | |
| | | | | | | | | cta | | | | | | | | 1880 |
| | Leu | Met | Thr | Lys | | Leu | Ile | Leu | Leu | Leu | Leu | Pro | Ser | Gln | Lys | |
| 435 | | | | | 440 | | | | | 445 | | | | | 450 | |
| | | | | | | | | gaa | | | | | | | | 1928 |
| His | Leu | Ile | Arg | | Ile | Ser | Ser | Glu | Pro | Met | Thr | Pro | Lys | Ser | Phe | |
| | | | | 455 | | | | | 460 | | | | | 465 | | |
| | | | | | | | | caa | | | | | | | | 1976 |
| тте | vaı | Pro | | Leu | Asp | Ser | Thr | Gln | Asp | Ser | Glu | Ala | | Leu | Gly | |
| | | | 470 | | | | | 475 | | | | | 480 | | | |

| | cga | cat | gta | ccc | cgt | CCC | cac | agt | ttg | cgg | atg | ctc | ctg | tca | acc | cca | 2024 |
|-------|------|-------|-------|-------|------|-------|-------|-------|-------|-------|------|-------|-------|-------|-------|-------|------|
| | | | | | | | | | | | | | | | Thr | | |
| | | | 485 | | | | | 490 | | | | | 495 | | | | |
| | tct | cac | acg | gta | cat | tac | tac | tgg | aga | aaa | ttt | gac | aat | gca | ttc | atg | 2072 |
| | | | | | | | | | | | | | | | Phe | | |
| | | 500 | | | | 505 | | | | | | 510 | | | | | |
| (| gt | cct | gtt | ttc | ggt | gga | cga | ggt | ttt | gta | cct | ttt | gtt | cca | gga | tca | 2120 |
| 2 | Arg | Pro | Val | Phe | Gly | Gly | Arg | Gly | Phe | Val | Pro | Phe | Val | Pro | Gly | Ser | |
| | 515 | | | | 520 | | | | | | 525 | | | | _ | 530 | |
| (| cct | act | gaa | ccg | gtc | gaa | ccg | acc | gaa | cca | aga | cca | gcc | gaa | tca | aga | 2168 |
| 1 | Pro | Thr | Glu | Pro | Val | Glu | Pro | Thr | Glu | Pro | Arg | Pro | Ala | Glu | Ser | Arq | |
| | | | | | 535 | | | | | 540 | | | | | 545 | , | |
| (| cca | acc | gaa | cca | act | gat | gag | tga- | ttaca | act o | gatg | gagat | g ca | aggti | tgcad | : | 2219 |
| ()1 | | | | Pro | | | | | | | | | | | - | | |
| (0 | | | | 550 | | | | | | | | | | | | | |
| | aaa | gtco | ca | ctggc | ctt | gg ag | gaag | gacga | a ago | gcagt | tttt | ttgc | gttt | ga d | aqttt | tgttt | 2279 |
| 17 | ctg | rttaa | ıta ç | gtttt | cgaa | at gt | ggtt | aaaa | a aaq | gggti | gtc | tagt | tttt | at a | atata | ggtcg | 2339 |
| iuc | aga | taco | rta a | attto | agct | c ag | jttco | cga | g gt | gaaco | cct | taga | ıggtt | tt o | cttcc | tgacg | 2399 |
| والما | ıttt | ttct | tc t | tttt | tgta | a tt | tato | aaaa | a aca | accaa | aatg | ggto | tata | itt o | ttta | agctt | 2459 |
| 1119 | ıtag | ctta | at t | acct | tata | a go | atgt | ggta | a gc | jttc | gtgt | aata | itgta | ıaa a | attto | cattg | 2519 |
| 10 | | | | aactt | | | | | | | | | - | | | _ | 2553 |
| - | 210 | > | 17 | 7 | | | | | | | | | | | | | |
| } ± < | 211 | > | 55 | 3 | | | | | | | | | | | | | |
| | 212 | | PF | Р | | | | | | | | | | | | | |
| < | 213 | > | Ni | erem | berg | ria h | ybri | .da | | | | | | | | | |
| < | 223 | > | Απ | nino | acid | seq | uenc | e of | pro | teir | reg | ulat | ing | the | рН | | |
| | | | of | vac | uole | s | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | |
| | 400 | | 17 | | | | | | | | | | | | | | |
| М | et. | Ala | Phe | Asp | | Gly | Thr | Leu | Leu | Gly | Lys | Met | Asn | Asn | Leu | Thr | |
| | | _ | | | 5 | | | | | 10 | | | | | 15 | | |
| Т | nr | ser | Asp | | Gln | Ser | Val | Val | | Val | Asn | Leu | Phe | Val | Ala | Leu | |
| | | | | 20 | | | | | 25 | | | | | 30 | | | |

| | Ile | Cys | | Cys | Ile | Val | Ile | | His | Leu | Leu | Glu | Glu | Asn | Arg | Trp |
|----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| | | | 35 | | | | | 40 | | | | | 45 | | | |
| | Met | Asn | Glu | Ser | Ile | Thr | Ala | Leu | Val | Ile | Gly | Ser | Cys | Thr | Gly | Val |
| | | 50 | | | | | 55 | | | | | 60 | | | | |
| | Ile | Ile | Leu | Leu | Ile | Ser | Gly | Gly | Lys | Asn | Ser | His | Ile | Leu | Val | Phe |
| | 65 | | | | | 70 | | | | | 75 | | | | | 80 |
| | Ser | Glu | Asp | Leu | Phe | Phe | Ile | Tyr | Leu | Leu | Pro | Pro | Ile | Ile | Phe | Asn |
| | | | | | 85 | | | | | 90 | | | | | 95 | |
| | Ala | Gly | Phe | Gln | Val | Lys | Lys | Lys | Ser | Phe | Phe | Arg | Asn | Phe | Ser | Thr |
| | | | | 100 | | | | | 105 | | | | | 110 | | |
| | Ile | Met | Leu | Phe | Gly | Ala | Val | Gly | Thr | Leu | Ile | Ser | Phe | Ile | Ile | Ile |
| | | | 115 | | | | | 120 | | | | | 125 | | | |
| | Ser | Ala | Gly | Ala | Ile | Gly | Ile | Phe | Lys | Lys | Met | Asp | Ile | Gly | His | Leu |
| 1,00 | | 130 | | | | | 135 | | | | | 140 | | - | | |
| 10 | Glu | Ile | Gly | Asp | Tyr | Leu | Ala | Ile | Gly | Ala | Ile | Phe | Ala | Ala | Thr | Asp |
| 4 | 145 | | | | | 150 | | | | | 155 | | | | | 160 |
| Se Se | Ser | Val | Cys | Thr | Leu | Gln | Val | Leu | Asn | Gln | Glu | Glu | Thr | Pro | Leu | Leu |
| | | | | | 165 | | | | | 170 | | | | | 175 | |
| The same | Ī | | | | | | | | | | | | | | | |
| 7007 | Tyr | Ser | Leu | Val | Phe | Gly | Glu | Gly | Val | Val | Asn | Asp | Ala | Thr | Ser | Val |
| 100 | | | | 180 | | | | | 185 | | | - | | 190 | | |
| 100 | Val | Leu | Phe | Asn | Ala | Val | Gln | Asn | Phe | Asp | Leu | Ser | His | Ile | Ser | Thr |
| 1 200 | l . | | 195 | | | | | 200 | | | | | 205 | | | |
| - Page | Gly | Lys | Ala | Leu | Gln | Leu | Ile | Gly | Asn | Phe | Leu | Tyr | Leu | Phe | Ala | Ser |
| | | 210 | | | | | 215 | | | | | 220 | | | | |
| | Ser | Thr | Phe | Leu | Gly | Val | Ala | Val | Gly | Leu | Leu | Ser | Ala | Phe | Ile | Ile |
| | 225 | | | | | 230 | | | | | 235 | | | | | 240 |
| | Lys | Lys | Leu | Tyr | Phe | Gly | Arg | His | Ser | Thr | Asp | Arg | Glu | Val | Ala | Ile |
| | | | | | 245 | | | | | 250 | | _ | | | 255 | |
| | Met | Ile | Leu | Met | Ala | Tyr | Leu | Ser | Tyr | Met | Leu | Ala | Glu | Leu | Phe | Tvr |
| | | | | 260 | | | | | 265 | | | | | 270 | | - |
| | Leu | Ser | Gly | Ile | Leu | Thr | Val | Phe | Phe | Cys | Gly | Ile | Val | Met | Ser | His |
| | | | 275 | | | | | 280 | | | | | 285 | | | |
| | Tyr | Thr | Trp | His | Asn | Val | Thr | Glu | Ser | Ser | Arg | Val | Thr | Thr | Lys | His |
| | | 290 | | | | | 295 | | | | | 300 | | | - | |

| Thr | Phe | Ala | Thr | Leu | Ser | Phe | Ile | Ala | Glu | Ile | Phe | Ile | Phe | Leu | Tyr |
|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|-------|------|-------|--------|--------|
| 305 | | | | | 310 | | | | | 315 | | | | | 320 |
| Val | Gly | Met | Asp | Ala | Leu | Asp | Ile | Glu | Lys | Trp | Lys | Phe | Val | Ser | Asp |
| | | | | 325 | | | | | 330 | | | | | 335 | |
| Ser | Pro | Gly | Thr | Ser | Ile | Lys | Val | Ser | Ser | Ile | Leu | Leu | Gly | Leu | Val |
| | | | 340 | | | | | 345 | | | | | 350 | | |
| Leu | Val | Gly | Arg | Gly | Ala | Phe | Val | Phe | Pro | Leu | Ser | Phe | Leu | Ser | Asn |
| | | 355 | | | | | 360 | | | | | 365 | | | |
| Leu | Thr | Lys | Lys | Asn | Pro | Glu | Asp | Lys | Ile | Ser | Phe | Asn | Gln | Gln | Val |
| | 370 | | | | | 375 | | | | | 380 | | | | |
| | | | | | | | | | | | | | | | |
| Thr | Ile | Trp | Trp | Ala | Gly | Leu | Met | Arg | Gly | Ala | Val | Ser | Met | Ala | Leu |
| 385 | | | | | 390 | | | _ | - | 395 | | | | | 400 |
| DAla | Tyr | Asn | Gln | Phe | Thr | Arg | Gly | Glv | His | | Gln | Leu | Ara | Ala | |
| 10 | | | | 405 | | _ | - | - | 410 | | | | 9 | 415 | |
| \⊍Ala | Ile | Met | Ile | Thr | Ser | Thr | Ile | Thr | | Val | Leu | Phe | Ser | | Val |
| 13 | | | 420 | | | | | 425 | | | | | 430 | | , 41 |
| // Val | Phe | Gly | Leu | Met | Thr | Lvs | Pro | | Ile | Leu | T.e11 | T.eu | | Pro | Sor |
| £U. | | 435 | | | | | 440 | | | | | 445 | Leu | 110 | Del |
| Gln | Lys | His | Leu | Ile | Arq | Met | | Ser | Ser | G111 | Pro | | Thr | Pro | T.17 C |
| 1 200 | 450 | | | | - | 455 | | | | | 460 | | | 110 | 1175 |
| Ser | Phe | Ile | Val | Pro | Leu | Leu | Asp | Ser | Thr | G1n | | Ser | Glu | Ala | Asn |
| 11465 | | | | 47 | | | - | | 47 | | | | 014 | | 30 |
| Leu | Gly | Arg | His | Val | Pro | Arg | Pro | His | Ser | Leu | Ara | Met. | Leu | Leu | Ser |
| | | | | 485 | | - | | | 490 | | 5 | | | 495 | |
| Thr | Pro | Ser | His | Thr | Val | His | Tvr | Tvr | Tro | Ara | T.VS | Phe | Asn | Asn | Δla |
| | | | 500 | | | | - | 505 | | 5 | -10 | | 510 | 11.511 | 2114 |
| Phe | Met | Arg | Pro | Val | Phe | Glv | Glv | | Glv | Phe | Val | Pro | | Val | Pro |
| | | 515 | | | | - | 520 | , | 2 | | | 525 | - 110 | | |
| Gly | Ser | Pro | Thr | Glu | Pro | Val | | Pro | Thr | Glu | Pro | | Pro | Ala | Glu |
| | 530 | | | | | 535 | | | | | 540 | 9 | -10 | niu | GIU |
| Ser | Arg | Pro | Thr | Glu | Pro | Thr | Asp | Glu | | | 5.0 | | | | |
| 545 | | | | | 550 | | | | | | | | | | |
| | | | | | | | | | | | | | | | |
| <210 |)> | 18 | 3 | | | | | | | | | | | | |
| <211 | .> | 23 | 61 | | | | | | | | | | | | |

| ~21 | 2- | L | MM | | | | | | | | | | | | | |
|-------|------|-----|--------------|------------|------|------|------|------|----------|----------|-------|------|------|------|-------|------|
| <21 | 3> | I | oren | ia h | ybri | .da | | | | | | | | | | |
| <22 | 3> | | ucle egul | | | | | | | | .ng f | or p | rote | ein | | |
| < 40 | 0> | 1 | .8 | | | | | | | | | | | | | |
| gtt | ggag | att | ccga | gctg | ca g | catc | acct | t gc | ttat | gtaa | gct | ttaa | aaq | tato | agaat | t 60 |
| | | | | | | | | | | | | | | | gtttg | |
| | | | | | | | | | | | | | | | gcgac | |
| | | | | | | | | | | | | | | | tgtct | |
| | | | | | | | | | | | | | | | gccca | |
| | | | | | | | | | | | | | | | aagat | |
| gaa | | | | | | | | | | | | | | | | 413 |
| 10 | | | | | | | | | | Met | Gly | Phe | Glu | Ser | Val | |
| [1] | | | | | | | | | | | | | | 5 | | |
| att | aag | cta | gcg | gca | agt | gaa | act | gac | aat | ttg | tgg | agc | tct | ggt | cac | 461 |
| (UIle | Lys | Leu | Ala | Ala | Ser | Glu | Thr | Asp | Asn | Leu | Trp | Ser | Ser | Gly | His | |
| 4.5 | | | 10 | | | | | 15 | | | | 20 | | | | |
| agt | | | | | | | | | | | | | | | | 509 |
| Gly | Ser | | Val | Ala | Ile | Thr | Leu | Phe | Val | Thr | Leu | Leu | Cys | Thr | Cys | |
| ,E | | 25 | | | | | 30 | | | | | 35 | | | | |
| []ata | gtg | att | ggt | cat | ctt | ctg | gag | gaa | aac | cgt | tgg | atg | aat | gaa | tct | 557 |
| i*Ile | Val | TTE | GTĀ | His | Leu | | Glu | Glu | Asn | Arg | | Met | Asn | Glu | Ser | |
| 240 | 40 | | | | | 45 | | | | | 50 | | | | | |
| ate | att | gcc | ctc | ata | att | ggt | tta | gcc | acg | gga | gtt | ata | atc | ctg | tta | 605 |
| 55 | TTE | Ата | Leu | TTE | | Gly | Leu | Ala | Thr | | Val | Ile | Ile | Leu | Leu | |
| | 200 | | | | 60 | | | | | 65 | | | | | 70 | |
| Tlo | cor | ggt | gga | aaa | agc | tcc | cat | ctc | ttg - | gtg | ttc | agt | gag | gat | ctt | 653 |
| TIE | ser | GTĀ | Gly | | Ser | ser | His | Leu | | Val | Phe | Ser | Glu | | Leu | |
| | | | | 75 | | | | | 80 | | | | | 85 | | |
| ttc | ++c | a+c | + = + | aac | a+ c | | | | | | | | | | | |
| Phe | Pho | Tla | tat | ycg N1a | tou | cca | cca | atc | att | ttt - | aat | gcg | aaa | ttc | caa | 701 |
| 1116 | 1116 | -T6 | Tyr 90 | urg | ьеи | Pro | Pro | | TTE | Phe | Asn | Ala | | Phe | Gln | |
| | | | 90 | | | | | 95 | | | | | 100 | | | |

| _ | | | | | | | . cgc | uuc | CCC | . yca | act | aca | aty | aty | LLL | /45 |
|------------------|-----|-----|-----|-----|-----|-----|-------|-----|-----|-------|-----|-----|-----|-----|-----|------|
| Val | Lys | Lys | Lys | Ser | Phe | Phe | Arg | Asn | Phe | Ala | Thr | Ile | Met | Met | Phe | |
| | | 105 | | | | | 110 | | | | | 115 | | | | |
| gga | gca | gtt | ggt | acc | ttg | ata | tcc | ttc | ato | ato | att | tca | ctc | ggt | aca | 797 |
| | | | | | | | | | | | | | | | Thr | |
| | 120 | | | | | 125 | | | | | 130 | | | - | | |
| att | gca | ttc | ttc | ccc | aaa | atg | aac | atg | aga | ctt | gga | gtt | gga | gat | tat | 845 |
| | | | | | | | | Met | | | | | | | | |
| 135 | | | | | 140 | | | | | 145 | | | _ | _ | 150 | |
| ctt | gct | att | gga | gct | att | ttt | gct | gca | aca | gac | tca | gtt | tgc | aca | tta | 893 |
| | | | | | | | | Ala | | | | | | | | |
| | | | | 155 | | | | | 160 | | | | _ | 165 | | |
| cag | gtg | cta | agc | cag | gac | gaa | aca | cca | ctg | ttg | tac | agt | cta | gtg | ttt | 941 |
| Gln | | | | | | | | | | | | | | | | |
| o o | | | 170 | | | | | 175 | | | | | 180 | | | |
| ggc | gag | ggt | gtt | gta | aat | gac | gcg | act | tca | gtg | gtc | cta | ttt | aat | gca | 989 |
| Gly | Glu | Gly | Val | Val | Asn | Asp | Ala | Thr | Ser | Val | Val | Leu | Phe | Asn | Ala | |
| ¥ | | 185 | | | | | 190 | | | | | 195 | | | | |
| [⊍] gta | cag | aac | ttc | gac | ctg | cct | cat | atg | tct | act | gct | aaa | gct | ttc | gag | 1037 |
| _Val | Gln | Asn | Phe | Asp | Leu | Pro | His | Met | Ser | Thr | Ala | Lys | Ala | Phe | Glu | |
| | 200 | | | | | 205 | | | | | 210 | | | | | |
| ctt | gtt | gga | aac | ttc | ttt | tat | tta | ttt | gct | aca | agc | act | gtg | ctg | ggt | 1085 |
| Leu | Val | Gly | Asn | Phe | Phe | Tyr | Leu | Phe | Ala | Thr | Ser | Thr | Val | Leu | Gly | |
| 215 | | | | 220 | | | | | | 225 | | | | | 230 | |
| | | | | | | | | tac | | | | | | | | 1133 |
| Val | Leu | Thr | Gly | Leu | Leu | Ser | Ala | Tyr | Ile | Ile | Lys | Lys | Leu | Tyr | Phe | |
| | | | | 235 | | | | | 240 | | | | | 245 | | |
| gga | agg | cac | tcc | act | gat | cgc | gag | gtt | gcc | ata | atg | ata | ctc | atg | gct | 1181 |
| Gly | Arg | His | Ser | Thr | Asp | Arg | Glu | Val | Ala | Ile | Met | Ile | Leu | Met | Ala | |
| | | | 250 | | | | | 255 | | | | | 260 | | | |
| | | | | | | | | tta | | | | | | | | 1229 |
| Tyr | Leu | | Tyr | Met | Leu | Ala | Glu | Leu | Phe | Asp | Leu | Ser | Gly | Ile | Leu | |
| | | 265 | | | | | 270 | | | | | 275 | | | | |
| acc | gtg | ttc | ttc | tgt | gga | att | gtg | atg | tcg | cac | tat | aca | tgg | cac | aat | 1277 |
| Thr | | Phe | Phe | Cys | Gly | | Val | Met | Ser | His | Tyr | Thr | Trp | His | Asn | |
| | 280 | | | | | 285 | | | | | 290 | | | | | |

| gto | act | gaa | aac | tca | aga | gtt | acc | acc | aag | cat | aca | ttt | gcg | aca | ttq | 1325 |
|--------------|------------|-----|-------|-----|------------|------|------------|------|------------|-------|-----|----------|-------|-------|-----|------|
| | Thr | | | | | | | | | | | | | | | |
| 295 | | | | | 300 | | | | | 305 | | | | | 310 | |
| tca | ttt | gtt | gct | gaa | ata | ttt | ata | ttt | ctg | tat | gtt | ggc | atq | gat | qct | 1373 |
| | Phe | | | | | | | | | | | | | | | |
| | | | | 315 | | | | | 320 | - | | - | | 325 | | |
| tta | gac | att | gag | aaa | tgg | aga | ttc | gta | agc | qqc | agc | atq | aca | aca | tct | 1421 |
| | Asp | | | | | | | | | | | | | | | |
| | | | 330 | | | | | 335 | | - | | | 340 | | | |
| gca | gCt | gtc | agt | gca | act | ctq | ctq | qqa | tta | att | tta | ctc | t.ca | aga | gca | 1469 |
| Ala | Ala | Val | Ser | Ala | Thr | Leu | Leu | Glv | Leu | Val | Leu | Leu | Ser | Ara | Ala | -103 |
| | | 345 | | | | | 350 | | | | | 355 | 001 | 9 | | |
| gcc | ttt | qta | ttc | cct | tta | tca | | ctc | tee | aat | C+a | | 222 | aar | tcc | 1517 |
| Ala | | | | | | | | | | | | | | | | 1317 |
| NU. | 360 | | | | | 365 | | | | ***** | 370 | | 2,0 | Lys | DCI | |
| (Q (U cca | ctc | gaa | aaa | atc | agt | | add | cad | caa | 2++ | | 2+2 | + ~ ~ | + ~ ~ | ~~+ | 1565 |
| Pro | Leu | Glu | Lvs | Ile | Ser | Leu | Ara | Gln | Gln | Tle | Tle | Tle | Trn | Trn | 712 | 1565 |
| [U 375 | | | -1- | | 380 | Dea | 1119 | OIII | GIII | 385 | 116 | TTE | пр | пр | 390 | |
| ggt | ctt | ato | cac | aaa | | a++ | +cc | a+~ | ac+ | | aat | + 2.0 | | | | 1613 |
| Gly | Leu | Met | Ara | Glv | Ala | Val | Ser | Mo+ | Ala Ala | Ton | yc. | m | aag | cag | 77. | 1613 |
| Part I | | | 9 | 395 | nia | val | per | nec | 400 | теп | Ald | Tyr | ьys | | Pne | |
| Wact. | aga | αаа | aat | | aca | a+a | ~~~ | aa+ | | 204 | | | | 405 | | |
| act | Ara | Glu | Glu | LOU | Thr | 1721 | gaa Clu | n | gaa | aat | gcc | ata | ttc | atc | acc | 1661 |
| 100 | 9 | O_u | 410 | пец | TIII | vaı | GIU | 415 | GIU | Asn | Ala | ше | | TTE | Thr | |
| agt | aca | 2+0 | | 2++ | ~+~ | a+a | | | | | | | 420 | | | |
| Ser | aca Thr | Tlo | Thr | Tlo | y Ly | Tou | Db. | age | act | gtg | gtg | ttt - | ggt | ttg | atg | 1709 |
| DCI | Thr | 425 | TIIL | TTE | Val | ьец | 430 | ser | Thr | Val | Val | | GLy | Leu | Met | |
| aca | 224 | | a+a | 2+4 | | | | | | | | 435 | | | | |
| Thr | aag | Pro | TOU | Tlo | aat aar | Tan | | ata | - | tca | cca | aag | ctt | aac | aga | 1757 |
| | Lys | 110 | пец | TTE | ASII | | Leu | тте | Pro | ser | | Lys | Leu | Asn | Arg | |
| +00 | | + | ± m n | | | 445 | | | | | 450 | | | | | |
| Com | gtc | 0 | cca | gaa | ccg | ctg | act | cca | aac | tcc | atc | aca | atc | cca | ctt | 1805 |
| | Val | ser | ser | GIu | | Leu | Thr | Pro | Asn | | Ile | Thr | Ile | Pro | Leu | |
| 455 | | | | | 460 | | | | | 465 | | | | | 470 | |
| CEC | ggg | gaa | agt | cag | gac | tct | gtg | gcc | gaa | cta | ttc | agc | atc | aga | ggt | 1853 |
| Leu | Gly | GLu | ser | | Asp | Ser | Val | Ala | | Leu | Phe | Ser | Ile | Arg | Gly | |
| | | | | 475 | | | | | 480 | | | | | 485 | | |

| | caa | act | tca | caa | ggt | ggc | gaa | CCC | gtt | gct | cga | ccg | agc | ago | cta | cqc | 1901 |
|-----|---|---|-------|------|-------|------|------|-------|------|-------|-------|------|-------|------|-------------------|--------|------|
| | | | Ser | | | | | | | | | | | | | | |
| | | | | 490 | | | | | 495 | | | | | 500 | | , | |
| | atg | tta | ctc | aca | aag | ccc | act | cat | acg | gtg | cac | tat | tat | tgg | aga | aaa | 1949 |
| | Met | Leu | Leu | Thr | Lys | Pro | Thr | His | Thr | Val | His | Tyr | Tyr | Trp | Ara | Lvs | |
| | | | 505 | | | | | 510 | | | | - | 515 | - | | - | |
| | ttc | gac | aat | gct | ttt | atg | cgt | ccg | gtc | ttt | ggt | ggg | cqt | qqc | ttt | qta | 1997 |
| | Phe | Asp | Asn | Ala | Phe | Met | Arg | Pro | Val | Phe | Gly | Gly | Arg | Gly | Phe | Val | |
| | | 520 | | | | | 525 | | | | | 530 | | - | | | |
| | cca | tat | gtt | ccc | ggt | tca | ccg | act | gaa | cga | agc | gtt | cgc | aac | tgg | gaa | 2045 |
| | Pro | Tyr | Val | Pro | Gly | Ser | Pro | Thr | Glu | Arg | Ser | Val | Arg | Asn | Trp | Glu | |
| | 535 | | | | | 540 | | | | | 545 | | | | - | 550 | |
| | gaa | gag | acc | aaa | cag | taaa | aaag | att t | tctt | gtgt | gaa | tgat | ggt | a a | gaga [.] | ttag | 2100 |
| | Glu | | Thr | | | | | | | | | | | | - | - | |
| 10 | | | | | 555 | | | | | | | | | | | | |
| Ęų | attetttgga tattegtttt tettatttet aatgtgteac etgggaagtt gttgaatgaa 216 | | | | | | | | | | | | | | 2160 | | |
| 12 | att | atat | tat o | gtc | tggtt | t to | gact | ttgc | gat | tgtg | gaa | ggaa | itatt | tc | ttct | ggattt | 2220 |
| 21 | tgc | attatattat cgtctggttt togactttgc gottgtggaa ggaatattto ttotggai tgcatggaaa cotcaatgat agggggtgtg atatttttgt tagaaactga gtogttt | | | | | | | | | | | ttgat | 2280 | | | |
| i. | gtatattgtt ggtaatgcag ctgggttttg ttttgtatgt atagtcatca agtg | | | | | | | | | | gtatt | 2340 | | | | | |
| 100 | tat | tcata | att o | gtta | tgcag | jt c | | | | | | | | | | , | 2361 |
| 111 | | | | | | | | | | | | | | | | | |
| 10 | <210 | 0> | 19 |) | | | | | | | | | | | | | |
| | <21 | | 5.5 | 55 | | | | | | | | | | | | | |
| 1 = | <212 | 2> | PF | PΤ | | | | | | | | | | | | | |
| | <213 | 3> | To | reni | ia hy | brid | la | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | |
| | <223 | 3> | An | ino | acid | seq | uenc | e of | pro | tein | reg | ulat | ing | the | рН | | |
| | | | of | vac | cuole | s | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | |
| | <400 | | 19 | | | | | | | | | | | | | | |
| | Met | Gly | Phe | Glu | | Val | Ile | Lys | Leu | Ala | Ala | Ser | Glu | Thr | Asp | Asn | |
| | | | | | 5 | | | | | 10 | | | | | 15 | | |
| | Leu | Trp | Ser | | Gly | His | Gly | Ser | Val | Val. | Ala | Ile | Thr | Leu | Phe | Val | |
| | | _ | _ | 20 | | | | | 25 | | | | | 30 | | | |
| | rhr | Leu | Leu | Cys | Thr | Cys | Ile | Val | Ile | Gly : | His : | Leu | Leu | Glu | Glu | Asn | |
| | | | 35 | | | | | 40 | | | | | 45 | | | | |

| Arg | | | Asn | Glu | Ser | Ile | Ile | Ala | Leu | Ile | Ile | Gly | Leu | Ala | Thr |
|--------|-------|-----|-----|-----|-----|-----|-----|-----|-----|------|-----|------|-------|--------|-------|
| | 50 | | | | | 55 | | | | | 60 | | | | |
| Gl | v Val | Ile | lle | Leu | Leu | Ile | Ser | Gly | Gly | Lys | Ser | Ser | His | Leu | Lev |
| 65 | ; | | | | 70 | | | | | 75 | | | | | 80 |
| Val | . Phe | Ser | Glu | Asp | Leu | Phe | Phe | Ile | Tyr | Ala | Leu | Pro | Pro | Ile | Ile |
| | | | | 85 | | | | | 90 | | | | | 95 | |
| Ph∈ | Asn | Ala | Gly | Phe | Gln | Val | Lys | Lys | Lys | Ser | Phe | Phe | Arg | Asn | Phe |
| | | | 100 | | | | | 105 | | | | | 110 | | |
| Ala | Thr | Ile | Met | Met | Phe | Gly | Ala | Val | Gly | Thr | Leu | Ile | Ser | Phe | Ile |
| | | 115 | | | | | 120 | | - | | | 125 | | | |
| Ile | Ile | Ser | Leu | Gly | Thr | Ile | Ala | Phe | Phe | Pro | Lys | Met | Asn | Met | Ara |
| | 130 | | | | | 135 | | | | | 140 | | | | , |
| Leu | Gly | Val | Gly | Asp | Tyr | Leu | Ala | Ile | Gly | Ala | Ile | Phe | Ala | Ala | Thr |
| 13 145 | | | | | 150 | | | | | 155 | | | | | 160 |
| Asp | Ser | Val | Cys | Thr | Leu | Gln | Val | Leu | Ser | Gln | Asp | Glu | Thr | Pro | Leu |
| 10 | | | | 165 | | | | | 170 | | | | | 175 | |
| Leu | Tyr | Ser | Leu | Val | Phe | Gly | Glu | Glv | Val | Val | Asn | Asp | Ala | Thr | Ser |
| 10 | | | 180 | | | - | | 185 | | | | | 190 | | DOL |
| Wal | Val | Leu | Phe | Asn | Ala | Val | Gln | Asn | Phe | Asp | Leu | Pro | His | Met | Ser |
| 17 | | 195 | | | | | 200 | | | | | 205 | | 1100 | DCI |
| Thr | Ala | Lys | Ala | Phe | Glu | Leu | Val | Glv | Asn | Phe | Phe | | T.en | Phe | Δla |
| 112 | 210 | | | | | 215 | | - | | | 220 | -2- | | | |
| Thr | Ser | Thr | Val | Leu | Gly | Val | Leu | Thr | Glv | Leu | | Ser | Ala | Tvr | Tle |
| 1-225 | | | | | 230 | | | | - | 235 | | | | -1- | 240 |
| Ile | Lys | Lys | Leu | Tyr | Phe | Gly | Arg | His | Ser | | Asp | Ara | Glu | Val | |
| | | | | 245 | | - | _ | | 250 | | | 5 | O.L.u | 255 | mu |
| | | | | | | | | | | | | | | 233 | |
| Ile | Met | Ile | Leu | Met | Ala | Tyr | Leu | Ser | Tvr | Met. | Leu | Ala | Glu | T.e.11 | Dho |
| | | | 260 | | | - | | 265 | -1- | | | | 270 | Leu | 1 110 |
| Asp | Leu | Ser | Gly | Ile | Leu | Thr | Val | | Phe | Cvs | Glv | Tle | | Mo+ | Sor |
| | | 275 | - | | | | 280 | | | 2,3 | 3-1 | 285 | · 41 | .160 | Set |
| His | Tyr | Thr | Trp | His | Asn | Val | | Glu | Asn | Ser | Ara | | Thr | Thr | Larc |
| | 290 | | - | | | 295 | | | | | 300 | ٧٩٢ | -111 | * *** | пуs |
| His | Thr | Phe | Ala | Thr | Leu | | Phe | Val | Ala | Glu | | Dhe | Tle | Phe | Tou |
| 305 | | | | | 310 | | | | | 315 | -16 | 1116 | -TG | EIIG | 320 |
| | | | | | | | | | | | | | | | |

| Туг | · Val | . Gly | Met | Asp 325 | | Leu | Asp | Ile | Glu 330 | | Trp | Arg | Phe | Val | |
|-------------------|------------|------------|------------|------------|------------|------------|-----|------------|------------|------------|------------|-----|------------|-----|-----|
| Gly | Ser | Met | Thr | Thr | Ser | Ala | Ala | Val | Ser | Ala | Thr | Leu | Leu 350 | Gly | |
| Val | Leu | Leu 355 | | Arg | Ala | Ala | Phe | Val | Phe | Pro | Leu | Ser | Phe | | Ser |
| Asn | Leu 370 | | Lys | Lys | Ser | Pro 375 | Leu | Glu | Lys | Ile | Ser 380 | Leu | Arg | Gln | Gln |
| 385 | | | | Trp | 390 | | | | | 395 | | | | | 400 |
| | | | | Gln 405 | | | | | 410 | | | | | 415 | |
| 523 | | | 420 | Ile | | | | 425 | | | | | 430 | | |
| Val | | 435 | | | | | 440 | | | | | 445 | | | |
| U U U | Pro 450 | Lys | Leu | Asn | Arg | Ser 455 | Val | Ser | Ser | Glu | Pro 460 | Leu | Thr | Pro | Asn |
| Ser - 465 | | | | | 470 | | | | | 475 | | | | | 400 |
| Leu (J | | | | 485 | | | | | 490 | Gly | | | | 495 | Ala |
| ^{1≜} Arg | Pro | Ser | Ser 500 | Leu | Arg | Met | Leu | Leu 505 | Thr | Lys | Pro | Thr | His | Thr | Val |
| | | 515 | | Arg | | | 520 | | | | | 525 | | | |
| | 530 | | | Phe | | 535 | | | | | Ser 540 | Pro | Thr | Glu | Arg |
| Ser 545 | Val | Arg | Asn | Trp | Glu 550 | Glu | Glu | Thr | Lys | Gln 555 | | | | | |
| <210 | 1> | 20 | | | | | | | | | | | | | |
| <211 | | | | | | | | | | | | | | | |
| | <212> | | A | | | | | | | | | | | | |
| <213> | | Ipomea nil | | | | | | | | | | | | | |

<223> Nucleotide sequence of promoter region of gene encoding for protein regulating the pH of vacuoles

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gctggatgaa gatgattcac tgcactttgg ttgcatattg aagctgcgat tgctatcgaa 1860

aacaaataac ctatctatat aaaacaaacg acttagattt agggaataag aaggaagata 1920 cttttttaa aatcccaaaa ttacctttta ggtttgacct gcaaataaca ctttaagatc 1980 aaatcagata aaatgtcata atcaatgatc aaattgaata attttagtag tcgaggatca 2040 aattggtaaa atccccatag tcgagggact aaaccagtaa ttttctcgcg tttgaacgtt 2100 tgtccgaaaa ttggcattag cgatagctta attgagtttt tcaattctct aattttttaa 2160 attttgtttc ttcataaaat ccttcacttt ttcactttgc taatattttg ccgaatttat 2220 aatatttcca atttctaaag tagcagaacc ccagacgttg aactgccaat ttttttttt 2280 gtttttgttt tttgtttttt tatttcctta tccctccacc tcattttgaa qttaattatt 2340 attattaatt cattaatttt taaaatagag agactgcatt aacacaaaat tagccaatta 2400 ggtagcagaa ttaaatttaa acaaacaagt tggtttaatg taatttttgt caatttaatt 2460 tctctatttt tggacaaaaa ttaggtagac ttattaaatt aaataaacat gtttgtttaa 2520 ttttacttct tctacctaag tttgtgtcaa ttcagtctct ctattttata aattaatgaa 2580 tggttaaata taacttataa gtgcattgtg tccaaatgat cacaagagtt aggccaactt 2640 ficttttttcat ataggtgatt cttttttcga gtattacgta cacttcagtc ttgtcaacta 2700 acacttagaa tttagttgtc attttcgaac ataggtgtca actaagtttg gtatccacta 2760 ii tatagcacat gtattccaag agatttaatc tcattcatca tgacaacttc tctaccaatt 2820 ctttgctcaa tcttttagtt agcgaattcg ctatattatc ctataacttt cagtatagtc 2880 👫 tatgtetaga ettacegtta tacatattae taatttaeta tatgteette caattgegaa 3000 attgactatcg taatgcatac atattggaga tatatatttt ttctaggggt aaatgcaggt 3060 #tggatcgacc cattaggcct gccccaacgc aaactttttt tqtcqqqctt ttqcqqaccq 3120 gettgegggt tagaaaatac acageecaag eeegteeatg egggetegeg ggeettattt 3180 Caaaaaaaaa aaaaatacta cgtattattc tattatttta tattcaaata gtctaatata 3240 aataaataaa aaaatcgtgt ttgaaaatta ctttttttt tatatatatt ttttaaaatt 3300 atatatatat atatatata attatttata ttatttatat ttatgtttat atttaaatac 3420 gggcatggct cgtcggctgg tccgttaggt ccgctctttt qtaqqccatt tttttqtqtq 3480 accetaaate gteteacege gggacaagta tagggeaget tgeggaette ggteeatttt 3540 gacatatata tatatata tatatatat tatatatata tatatata tatatatat tatatatata 3600 tatatatata taacattaaa atttaaaaaa tatagatttt ttttaaacat gaaaaaaatt 3660 ggctggtcca taaaagcccg taaaaagaat acgttggggt tggcctaatg gaccgatcca 3780 accegeattg acacecatag gaaaaacate tateteeaat ttgtatgeat tacaatagte 3840 aatttgcaat tggaagagca tatagtaatt agtaatatgt ataacggtaa gtctagacat 3900 attgaacatg tacataatac tattaaatag cttcttgcaa taggttttat ttttgttgac 3960

tatacatgaa gttataggat aatatagcga attcacaaac taaaagattg agcaatgagt 4020

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001560-397.ST25



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SEQUENCE LISTING

<110> Iida, Shigeru Tanaka, Sachiko Inagaki, Yoshishige <120> Genes Encoding Proteins Regulating the pH of Vacuoles <130> 001560-397 <140> 09/830,123 <141> 2001-04-24 <150> PCT/JP00/05722 <151> 2000-08-24 <150> JP 11/236800 <151> 1999-08-24 <160> 20 <170> PatentIn version 3.1 <210> 1 <211> <212> DNA <213> Ipomoea nil <220> <221> misc feature <222> (1) ... (2237) <223> Nucleotide sequence of DNA encoding for protein regulating the pH of vacuoles <400> 1 agaatgtagg ctacagaaat tttcagacag atagatacat aaatccgtat aatagagaca gagaaacaga aaaagagaga gtcacgttaa teetgagatt tteeteeatt tgtctgaage tetteateet teaacactae coccacatet cacettteaa gtgatttgta tgtttteggg 180 agggattgga atgggcaacc cggatatgtg aacagaaacc acgacattgg gaaaagattt 240 attgcaaaaa ttgttttgat tgttttggat tttgtggtag aaaaagggga agaacaaaa 299 atg gcg ttc ggg ttg tct tct ttg ctc caa aat tcg gat ttg ttc acg 347 Met Ala Phe Gly Leu Ser Ser Leu Leu Gln Asn Ser Asp Leu Phe Thr

tgc gca tgc att gtt ctt ggc cat cta ctc gag gag aat cgc tgg gtg 443 Cys Ala Cys Ile Val Leu Gly His Leu Leu Glu Glu Asn Arg Trp Val 45

395

tot gat cat got too gtt gtg tog atg aac ote ttt gtg gog ttg ott Ser Asp His Ala Ser Val Val Ser Met Asn Leu Phe Val Ala Leu Leu

40

| aac Asn | gaa Glu 50 | tcc Ser | att Ile | act Thr | gcc Ala | ctt Leu 55 | ata Ile | att Ile | ggt Gly | ttg Leu | tgc Cys 60 | acc Thr | gga Gly | gtt Val | gta Val | 491 |
|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|------|
| att Ile 65 | ttg Leu | ctc Leu | ctt Leu | agc Ser | gga Gly 70 | gga Gly | aag Lys | agt Ser | tca Ser | cat His 75 | ctt Leu | ctc Leu | gtc Val | ttt Phe | agc Ser 80 | 539 |
| gaa Glu | gat Asp | ctt Leu | ttc Phe | ttt Phe 85 | ata Ile | tat Tyr | ctc Leu | ctg Leu | cca Pro 90 | cct Pro | ata Ile | ata Ile | ttc Phe | aat Asn 95 | gcg Ala | 587 |
| ggg Gly | ttt Phe | caa Gln | gtg Val 100 | aaa Lys | aag Lys | aag Lys | cag Gln | ttt Phe 105 | ttc Phe | gtg Val | aac Asn | ttc Phe | atg Met 110 | aca Thr | att Ile | 635 |
| atg Met | ctg Leu | ttt Phe 115 | gga Gly | gct Ala | att Ile | ggc Gly | aca Thr 120 | ctt Leu | att Ile | agc Ser | tgt Cys | tct Ser 125 | att Ile | ata Ile | tca Ser | 683 |
| ttt Phe | ggt Gly 130 | gcg Ala | gtc Val | aaa Lys | att Ile | ttc Phe 135 | aag Lys | cac His | tta Leu | gac Asp | att Ile 140 | gac Asp | ttt Phe | ctg Leu | gat Asp | 731 |
| ttt Phe 145 | gga Gly | gat Asp | tat Tyr | tta Leu | gca Ala 150 | att Ile | ggt Gly | gcg Ala | ata Ile | ttt Phe 155 | gct Ala | gca Ala | acc Thr | gat Asp | tct Ser 160 | 779 |
| gtt Val | tgc Cys | aca Thr | ttg Leu | cag Gln 165 | gtg Val | ctc Leu | agt Ser | cag Gln | gat Asp 170 | gag Glu | acg Thr | ccc Pro | cta Leu 175 | ctt Leu | tac Tyr | 827 |
| agt Ser | ctc Leu | gtg Val | ttt Phe 180 | gga Gly | gaa Glu | ggg Gly | gtc Val 185 | gtc Val | aat Asn | gat Asp | gct Ala | aca Thr | tct Ser 190 | gtg Val | gtc Val | 875 |
| ctt Leu | ttt Phe | aat Asn 195 | gct Ala | att Ile | caa Gln | agt Ser | ttt Phe 200 | gac Asp | atg Met | act Thr | agt Ser | ttt Phe 205 | gat Asp | cca Pro | aaa Lys | 923 |
| att Ile | ggg Gly 210 | ctt Leu | cat His | ttc Phe | att | gga Gly 215 | Asn | ttc Phe | ttg Leu | tat Tyr | tta Leu 220 | ttt Phe | ctc Leu | tog Ser | agc Ser | 971 |
| act Thr 225 | Phe | ttg Leu | ggc Gly | gtg Val | gga Gly 230 | Ile | gga Gly | ctg Leu | ctt Leu | tgt Cys 235 | Ala | tat Tyr | att | atc Ile | aaa Lys 240 | 1019 |
| aag Lys | cta Leu | tac Tyr | ttt Phe | ggc Gly 245 | Arg | cac | tca Ser | acc Thr | gat Asp 250 | Arg | gag Glu | gtt Val | gcc Ala | ctt Leu 255 | atg Met | 1067 |
| atg Met | ctc Leu | atg Met | tct Ser 260 | Tyr | ttg Leu | tct Ser | tat | ata Ile 265 | Met | gcc Ala | gag Glu | tta Leu | ttc Phe 270 | Tyr | cta Leu | 1115 |
| ago | gac | ata | ctt | act | gta | ttc | ttc | tgt | gga | att | gto | atg | tct | cat | tat | 1163 |

| Ser | Gly | Ile 275 | Leu | Thr | Val | Phe | Phe 280 | Cys | Gly | Ile | Val | Met 285 | Ser | His | Tyr | |
|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|------|
| | | | | gtt Val | | | | | | | | | | | | 1211 |
| ttt Phe 305 | gca Ala | act Thr | ctg Leu | tca Ser | ttt Phe 310 | gtc Val | gca Ala | gag Glu | aca Thr | ttt Phe 315 | atc Ile | ttc Phe | ctc Leu | tat Tyr | gtt Val 320 | 1259 |
| | | | | ttg Leu 325 | | | | | | | | | | | | 1307 |
| cag Gln | gga Gly | cta Leu | tca Ser 340 | gtt Val | gca Ala | gtg Val | agc Ser | tca Ser 345 | ata Ile | ttg Leu | gta Val | ggc Gly | cta Leu 350 | atc Ile | tta Leu | 1355 |
| gta Val | ggc Gly | aga Arg 355 | gct Ala | gcg Ala | ttc Phe | gta Val | ttc Phe 360 | ccc Pro | ttg Leu | tcg Ser | ttt Phe | tta Leu 365 | tcc Ser | aac Asn | tta Leu | 1403 |
| gca Ala | aag Lys 370 | aaa Lys | aac Asn | tct Ser | tcg Ser | gac Asp 375 | aag Lys | ata Ile | tcc Ser | ttt Phe | agg Arg 380 | caa Gln | caa Gln | ata Ile | ata Ile | 1451 |
| | | | | ggc Gly | | | | | | | | | | | | 1499 |
| tat Tyr | aat Asn | aag Lys | ttt Phe | aca Thr 405 | acc Thr | tcg Ser | ggg Gly | cat His | acg Thr 410 | tca Ser | ttg Leu | cac His | gag Glu | aac Asn 415 | gca Ala | 1547 |
| ata Ile | atg Met | att Ile | aca Thr 420 | agt Ser | act Thr | gtt Val | acg Thr | gtt Val 425 | gtt Val | ctg Leu | ttc Phe | agc Ser | aca Thr 430 | gtt Val | gta Val | 1595 |
| ttc Phe | ggg Gly | ttg Leu 435 | atg Met | acg Thr | aag Lys | cct Pro | ctg Leu 440 | ata Ile | aac Asn | ctt Leu | ctg Leu | cta Leu 445 | ccc Pro | ccg Pro | cac His | 1643 |
| aag Lys | cag Gln 450 | atg Met | cca Pro | agc Ser | ggt Gly | cat His 455 | tcg Ser | tca Ser | atg Met | aca Thr | aca Thr 460 | tcc Ser | gaa Glu | ccc Pro | agt Ser | 1691 |
| agt Ser 465 | Pro | aag Lys | cac | ttc Phe | acg Thr 470 | gtg Val | cca Pro | ctc Leu | ctg Leu | gac Asp 475 | aac Asn | caa Gln | cct Pro | gac Asp | tca Ser 480 | 1739 |
| gaa Glu | agc Ser | gat Asp | atg Met | ata Ile 485 | acc Thr | gga Gly | cct | gag Glu | gtt Val 490 | Ala | cga Arg | cca Pro | act Thr | gcc Ala 495 | ttg Leu | 1787 |
| cgc Arg | atg Met | ctg Leu | cta Leu | agg Arg | acg Thr | cca Pro | acc Thr | cac His | acc Thr | gtg Val | cac | cgc Arg | tac Tyr | tgg Trp | cgt Arg | 1835 |

505 510 1883 aag ttt gat gat tog ttt atg ogt ooc gtg ttt ggc ggg ogg gga ttc Lys Phe Asp Asp Ser Phe Met Arg Pro Val Phe Gly Gly Arg Gly Phe gtt cog ttt gtc geg ggc tca cca gtt gag cag agc cct aga tga 1928 Val Pro Phe Val Ala Gly Ser Pro Val Glu Gln Ser Pro Arg ggtacaaagt acaaacaaga cactgttgct gggtgaaata gtgtaagttg tatcatagtt 1988 gattctggtt gcccctctta tgaaatgggc tgggtgaaag tcttctcact agctaggttg 2048 cattgcattg ctacttcata aatgttttat tttattttgt aaatgttggt gcattttagg 2108 tacttgtatt aacacctcat ttgtagcata ttatttggta cagagtattt tttttatgaa 2168 2237 aaaaaaaaa <210> <211> 542 <212> PRT <213> Ipomea nil <220> <221> peptide <222> (1)..(542) Amino acid sequence of protein regulating the pH of vacuoles <223> <400> 2 Met Ala Phe Gly Leu Ser Ser Leu Leu Gln Asn Ser Asp Leu Phe Thr Ser Asp His Ala Ser Val Val Ser Met Asn Leu Phe Val Ala Leu Leu Cys Ala Cys Ile Val Leu Gly His Leu Leu Glu Glu Asn Arg Trp Val Asn Glu Ser Ile Thr Ala Leu Ile Ile Gly Leu Cys Thr Gly Val Val Ile Leu Leu Ser Gly Gly Lys Ser Ser His Leu Leu Val Phe Ser Glu Asp Leu Phe Phe Ile Tyr Leu Leu Pro Pro Ile Ile Phe Asn Ala Gly Phe Gln Val Lys Lys Gln Phe Phe Val Asn Phe Met Thr Ile

- Met Leu Phe Gly Ala Ile Gly Thr Leu Ile Ser Cys Ser Ile Ile Ser 115 120 125
- Phe Gly Ala Val Lys Ile Phe Lys His Leu Asp Ile Asp Phe Leu Asp 130 135
- Val Cys Thr Leu Gln Val Leu Ser Gln Asp Glu Thr Pro Leu Leu Tyr 165 170 175
- Ser Leu Val Phe Gly Glu Gly Val Val Asn Asp Ala Thr Ser Val Val 180 185 190
- Leu Phe Asn Ala Ile Gln Ser Phe Asp Met Thr Ser Phe Asp Pro Lys 195 200
- Ile Gly Leu His Phe Ile Gly Asn Phe Leu Tyr Leu Phe Leu Ser Ser 210 215 220
- Thr Phe Leu Gly Val Gly Ile Gly Leu Leu Cys Ala Tyr Ile Ile Lys 225 230 235 240
- Lys Leu Tyr Phe Gly Arg His Ser Thr Asp Arg Glu Val Ala Leu Met 245 250 250
- Met Leu Met Ser Tyr Leu Ser Tyr Ile Met Ala Glu Leu Phe Tyr Leu 260 270
- Ser Gly Ile Leu Thr Val Phe Phe Cys Gly Ile Val Met Ser His Tyr
- Thr Trp His Asn Val Thr Glu Ser Ser Arg Val Thr Thr Arg His Ser 290 295 300
- Phe Ala Thr Leu Ser Phe Val Ala Glu Thr Phe Ile Phe Leu Tyr Val 305 310 315
- Gly Met Asp Ala Leu Asp Ile Glu Lys Trp Lys Phe Val Lys Asn Ser
- Val Gly Arg Ala Ala Phe Val Phe Pro Leu Ser Phe Leu Ser Asn Leu 365
- Ala Lys Lys Asn Ser Ser Asp Lys Ile Ser Phe Arg Gln Gln Ile Ile
- Ile Trp Trp Ala Gly Leu Met Arg Gly Ala Val Ser Ile Ala Leu Ala 385 \$390\$
- Tyr Asn Lys Phe Thr Thr Ser Gly His Thr Ser Leu His Glu Asn Ala 405 410 415

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Ile Met Ile Thr Ser Thr Val Thr Val Val Leu Phe Ser Thr Val Val
 Phe Gly Leu Met Thr Lys Pro Leu Ile Asn Leu Leu Pro Pro His
 Lys Gln Met Pro Ser Gly His Ser Ser Met Thr Thr Ser Glu Pro Ser
 Ser Pro Lys His Phe Thr Val Pro Leu Leu Asp Asn Gln Pro Asp Ser
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 Glu Ser Asp Met Ile Thr Gly Pro Glu Val Ala Arg Pro Thr Ala Leu
            485
 Arg Met Leu Leu Arg Thr Pro Thr His Thr Val His Arg Tyr Trp Arg
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 Lys Phe Asp Asp Ser Phe Met Arg Pro Val Phe Gly Gly Arg Gly Phe
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Val Pro Phe Val Ala Gly Ser Pro Val Glu Gln Ser Pro Arg
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   530
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 tactcaggac tcat
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gatgagtcct gagtaa
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<223> TIR+N primer
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<221> misc feature
<222> (19)..(19)
<223> Nucleotide 19 = "n" wherein "n" = any nucleotide
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                                                                       19
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<210> 8
<211> 17
<212> DNA
<213> Artificial Sequence
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<223> MseI+N primer
 <220>
<221> misc_feature
<222> (17)..(17)
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<400> 8
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<210> 9
<211> 130
<212> DNA
 <213> Artificial Sequence
 <220>
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,E

| <223> | MseI+N primer | |
|----------------------------------|--|-----|
| <400> tgagcat | 9 httt tottgtagtg otgagattit cotocattig totgaagoto ticatcotto | 60 |
| aacacta | acce ceacatetea cettteaagg tecaatettt ateatteate tttaeteagg | 120 |
| actcato | egte | 130 |
| <210> <211> <212> <213> | 26 | |
| <220> <223> | PR-5 primer | |
| <400> gggatco | 10 caac aaaaatggct gtcggg | 26 |
| <211> <212> | 11 29 DNA Artificial Sequence | |
| <220> <223> | PR-3 primer | |
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| | 12 patot gggggtogac at | 22 |
| <210> <211> <212> <213> | 22 | |
| <220> <223> | CBSC2-linker | |
| | 13 | 22 |

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<211> 2423
<212> DNA
<213> Petunia hybrida
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<221> misc feature
<222> (1)..(2423)
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       pH of vacuoles
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                                                                   180
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ttt gat ttt ggg acg ttg ttg gga aat gta gac agg tta tcg aca tct
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Phe Asp Phe Gly Thr Leu Leu Gly Asn Val Asp Arg Leu Ser Thr Ser
gat cat caa toa gtt gtg tog ata aac tta ttc gtt gct ctt att tgc
                                                                   453
Asp His Gln Ser Val Val Ser Ile Asn Leu Phe Val Ala Leu Ile Cys
gcg tgt att gtg atc ggt cat ttg ttg gaa gaa aac aga tgg atg aat
Ala Cys Ile Val Ile Gly His Leu Leu Glu Glu Asn Arg Trp Met Asn
                     40
gag too ata act goo tta gtg att ggt tot tgt act gga atc gtt att
                                                                    549
Glu Ser Ile Thr Ala Leu Val Ile Gly Ser Cys Thr Gly Ile Val Ile
                                                                    597
cta ctg ata agt gga gga aag aac tot cat att tta gtg tto agt gaa
Leu Leu Ile Ser Gly Gly Lys Asn Ser His Ile Leu Val Phe Ser Glu
                                                                    645
gat ctt ttc ttc att tac ctt ctt ccg cca atc att ttt aat gct ggg
Asp Leu Phe Phe Ile Tyr Leu Leu Pro Pro Ile Ile Phe Asn Ala Gly
                                                                    693
ttc cag gtg aaa aag aaa tcg ttc ttc cgc aat ttc agc act atc atg
Phe Gln Val Lys Lys Ser Phe Phe Arg Asn Phe Ser Thr Ile Met
ctc ttt ggg gca ctt ggc acc ttg ata tca ttc att att ata tca tta
                                                                    741
Leu Phe Gly Ala Leu Gly Thr Leu Ile Ser Phe Ile Ile Ser Leu
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| ggt Gly | gcc Ala | att Ile | ggc Gly | att Ile 135 | t t c Phe | aag Lys | aaa Lys | atg Met | aat Asn 140 | att Ile | gga Gly | agc Ser | ctt Leu | gaa Glu 145 | att Ile | 789 |
|-------------------|---------------------|---------------------|-------------------|--------------------|---------------------|-------------------|-------------------|-------------------|-------------------|--------------------|-------------------|-------------------|-------------------|-------------------|-------------------|------|
| gga Gly | gat Asp | tac Tyr | ctt Leu 150 | gca Ala | att Ile | ggg Gly | gca Ala | atc Ile 155 | ttc Phe | tct Ser | gct Ala | aca Thr | gat Asp 160 | tct Ser | gta Val | 837 |
| tgc Cys | acc Thr | tta Leu 165 | caa Gln | gtg Val | ctt Leu | aat Asn | cag Gln 170 | gat Asp | gaa Glu | aca Thr | ccc Pro | tta Leu 175 | ttg Leu | tac Tyr | agt Ser | 885 |
| cta Leu | gtt Val 180 | ttt Phe | ggg Gly | gaa Glu | ggt Gly | gtt Val 185 | gtg Val | aat Asn | gat Asp | gcc Ala | aca Thr 190 | tct Ser | gta Val | gtt Val | ctg Leu | 933 |
| ttc Phe 195 | aat Asn | gct A l a | atc Ile | cag Gln | aac Asn 200 | ttt Phe | gac Asp | tta Leu | tct Ser | cac His 205 | atc Ile | gac Asp | acg Thr | ggc Gly | aaa Lys 210 | 981 |
| gct Ala | atg Met | gaa Glu | tta Leu | gtt Val 215 | gga Gly | aac Asn | ttt Phe | cta Leu | tac Tyr 220 | ttg Leu | ttt Phe | gcc Ala | tca Ser | agc Ser 225 | act Thr | 1029 |
| gcc Ala | cta Leu | gga Gly | gtt Val 230 | gct Ala | gct Ala | ggc Gly | cta Leu | ctg Leu 235 | agc Ser | gcc Ala | tat Tyr | att Ile | att Ile 240 | aaa Lys | aaa Lys | 1077 |
| ctc Leu | tac Tyr | ttt Phe 245 | gga Gly | agg Arg | cac His | tca Ser | act Thr 250 | gac Asp | cgt Arg | gag Glu | gtt Val | gct Ala 255 | ata Ile | atg Met | ata Ile | 1125 |
| ctc Leu | atg Met 260 | Ala | tac Tyr | cta Leu | tct Ser | tac Tyr 265 | atg Met | ctt Leu | gct Ala | ga a Glu | tta Leu 270 | Phe | tat Tyr | tta Leu | agt Ser | 1173 |
| gca Ala 275 | Ile | ctc | act Thr | gt g Val | ttt Phe 280 | Phe | tct Ser | ggg Gly | atc | gtg Val 285 | Met | tct Ser | cac | tac Tyr | acc Thr 290 | 1221 |
| tgg Trp | cat | aat Asn | gtg Val | act Thr 295 | Glu | agc Ser | tcg Ser | aga Arg | gto Val 300 | Thr | Thr | aag Lys | cac His | act Thr 305 | Phe | 1269 |
| gct Ala | aca Thr | tta Leu | tca Ser 310 | | att | gct Ala | gaa Glu | ata Ile 315 | Phe | ata Ile | tto Phe | ctt Leu | tat Tyr 320 | Val | ggt Gly | 1317 |
| atg Met | gat Asp | gct Ala 325 | Let | gac Asp | att Ile | gag Glu | aag Lys 330 | Trp | aaç Lys | ttt Phe | gta Val | ago Ser 335 | : Asp | ago Ser | cct Pro | 1365 |
| gga Gly | ata / Ile 340 | Se: | gtt Val | cag Gln | gtt Val | ago Ser 345 | : Ser | ata : Ile | tto Lev | cto Let | ggt Gly 350 | / Let | gtt Val | tto Leu | gtt Val | 1413 |
| gga | a aga | a gca | a gca | a ttt | gtt | t tto | coa | ttg | , tca | tto | tto | g tco | aac | tto | acc | 1461 |

| Gly 355 | Arg | Ala | Ala | Phe | Val 360 | Phe | Pro | Leu | Ser | Phe 365 | Leu | Ser | Asn | Leu | Thr 370 | |
|------------|-------------------|------------|-------------------|------------|------------|-------------------|------------|-------------------|------------|------------|-------------------|------------|-------------------|-------------------|------------|------|
| | | | | | | | | | | | | | | aca Thr 385 | | 1509 |
| tgg Trp | tgg Trp | gct Ala | gga Gly 390 | ctt Leu | atg Met | aga Arg | ggt Gly | gcc Ala 395 | gtt Val | tct Ser | atg Met | gcc Ala | ctt Leu 400 | gct Ala | tat Tyr | 1557 |
| | | | | | | | | | | | | | | gca Ala | | 1605 |
| atg Met | atc Ile 420 | aca Thr | agt Ser | act Thr | atc Ile | act Thr 425 | gtt Val | gtc Val | ctt Leu | ttc Phe | agc Ser 430 | aca Thr | gtc Val | gtg Val | ttt Phe | 1653 |
| | | | | | | | | | | | | | | cac His | | 1701 |
| | | | | | | | | | | | | | | tcc Ser 465 | | 1749 |
| | | | | | | | | | | | | | | ctg Leu | | 1797 |
| | | | | | | | | | | | | | | acc Thr | | 1845 |
| | | | | | | | | | | | | | | ttc Phe | | 1893 |
| | | | | | | | | | | | | | | gga Gly | | 1941 |
| | | | | | | | | ttg Leu | | tga | tgga | gat | acag | attg | Ca . | 1991 |
| aaa | agtg | gtc | ttgg | tgag | gg a | agag | ggca | g tt | tttt | ggta | atg | aggt | tac | gttt | tcttta | 2051 |
| atg | ttaa | tag | caag | tgtg | gt t | aaaa | aggg | g tt | gtota | agtt | tat | aggt | ttt. | gcag | atctca | 2111 |
| agt | atat | tca | tttg | ggtg | at c | atgt | ttto | a gc | tcag | ttat | tgc | tttt | ggt | catt. | gctgac | 2171 |
| cat | caat | ttc | tgtg | ggga | at t | ccta | tagg | t tt | tete | ccta | aca | gttc | ttt | tctt | catctt | 2231 |
| ttt | gcaa | ttt. | atcg | aaac | ac c | aaat | gggt | g ta | tatt | ctgt | aag | cttg | tgg | cata | gctagc | 2291 |

ttaattgtot tgtaaaattt octacaggtt agagattggt tottgatatg tagatttoat 2351 atgattgtaa cattoccatt totcagaaaa gaaactataa tataaaattt otggtggotg 2411 tegeoogtge to 2423

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<220>

<221> peptide

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<223> Amino acid sequence of protein regulating the pH of vacuoles

<400> 15

Met Ala Phe Asp Phe Gly Thr Leu Leu Gly Asn Val Asp Arg Leu Ser 10 15

Thr Ser Asp His Gln Ser Val Val Ser Ile Asn Leu Phe Val Ala Leu 20 25 30

Ile Cys Ala Cys Ile Val Ile Gly His Leu Leu Glu Glu Asn Arg Trp \$35\$

Met Asn Glu Ser Ile Thr Ala Leu Val Ile Gly Ser Cys Thr Gly Ile $50 \hspace{1cm} 55 \hspace{1cm} 60 \hspace{1cm}$

Ser Glu Asp Leu Phe Phe Ile Tyr Leu Leu Pro Pro Ile Ile Phe Asn $85 \hspace{1.5cm} 90 \hspace{1.5cm} 95$

Ala Gly Phe Gln Val Lys Lys Lys Ser Phe Phe Arg Asn Phe Ser Thr $100 \,$ $\,$ $105 \,$

Ile Met Leu Phe Gly Ala Leu Gly Thr Leu Ile Ser Phe Ile Ile Ile 115 120 125

Ser Leu Gly Ala Ile Gly Ile Phe Lys Lys Met Asn Ile Gly Ser Leu 130 135 140

Glu Ile Gly Asp Tyr Leu Ala Ile Gly Ala Ile Phe Ser Ala Thr Asp 145 \$150\$

Ser Val Cys Thr Leu Gln Val Leu Asn Gln Asp Glu Thr Pro Leu Leu 165 \$170\$

Val Leu Phe Asn Ala Ile Gln Asn Phe Asp Leu Ser His Ile Asp Thr 195 200 205

Gly Lys Ala Met Glu Leu Val Gly Asn Phe Leu Tyr Leu Phe Ala Ser 210 215 220

Ser Thr Ala Leu Gly Val Ala Ala Gly Leu Leu Ser Ala Tyr Ile Ile 225 230 235

Lys Lys Leu Tyr Phe Gly Arg His Ser Thr Asp Arg Glu Val Ala Ile $245 \hspace{1.5cm} 250 \hspace{1.5cm} 250$

Met Ile Leu Met Ala Tyr Leu Ser Tyr Met Leu Ala Glu Leu Phe Tyr 260 265 270

Leu Ser Ala Ile Leu Thr Val Phe Phe Ser Gly Ile Val Met Ser His 275 280 285

Tyr Thr Trp His Asn Val Thr Glu Ser Ser Arg Val Thr Thr Lys His 290 295 300

Thr Phe Ala Thr Leu Ser Phe Ile Ala Glu Ile Phe Ile Phe Leu Tyr 305 \$310\$ \$315

Val Gly Met Asp Ala Leu Asp Ile Glu Lys Trp Lys Phe Val Ser Asp 325 330 335

Ser Pro Gly Ile Ser Val Gln Val Ser Ser Ile Leu Leu Gly Leu Val $340 \hspace{1.5cm} 345 \hspace{1.5cm} 345 \hspace{1.5cm} 350 \hspace{1.5cm}$

Leu Val Gly Arg Ala Ala Phe Val Phe Pro Leu Ser Phe Leu Ser Asn $355 \hspace{1.5cm} 360 \hspace{1.5cm} 365$

Leu Thr Lys Lys Thr Pro Glu Ala Lys Ile Ser Phe Asn Gln Gln Val $_{\rm 370}$ $_{\rm 370}$

Thr Ile Trp Trp Ala Gly Leu Met Arg Gly Ala Val Ser Met Ala Leu 385 \$390\$

Ala Tyr Asn Gln Phe Thr Arg Gly Gly His Thr Gln Leu Arg Ala Asn 405 410 415

Ala Ile Met Ile Thr Ser Thr Ile Thr Val Val Leu Phe Ser Thr Val 420 425 430

Val Phe Gly Leu Met Thr Lys Pro Leu Ile Arg Ile Leu Leu Pro Ser 435 440 445

His Lys His Leu Ser Arg Met Ile Ser Ser Glu Pro Thr Thr Pro Lys 450 455

Ser Phe Ile Val Pro Leu Leu Asp Ser Thr Gln Asp Ser Glu Ala Asp 465 470475

Leu Glu Arg His Val Pro Arg Pro His Ser Leu Arg Met Leu Leu Ser 485 490 495

Thr Pro Ser His Thr Val His Tyr Tyr Trp Arg Lys Phe Asp Asn Ala Phe Met Arg Pro Val Phe Gly Gly Arg Gly Phe Val Pro Phe Ala Pro Gly Ser Pro Thr Asp Pro Val Gly Gly Asn Leu Gln <210> 16 <211> 2553 <212> DNA <213> Nierembergia hybrida <221> misc feature <222> (1)..(2553) <223> Nucleotide sequence of DNA encoding for protein regulating the pH of vacuoles <400> 16 aattattatt atttctctcc aactctcatt tctcagtttg ttgtgacttt ttcagagctt 60 qaaqttcagt taattcattt tocaatatat tgattgtttt catttgagcg cgagaggatt togtottoto aatotgottt caaatoottt ttgtttgtga tattogatat tattoactoa 180 gtttacctta atatttcctc gcactttctg aattcgagtg ctttgaagtg tgttggattt 240 300 cgaaaagcqq aagaaaattc agcaaaaacg ctgttgctga atttgcagca gtttgagttt ttgctaaata gctaagatct gattgaattt ttcactggtg cttataggga aattcgacgt 360 cgttttgact gcaatatttg tccgtgattc ggactttgtt gaaattttgc tatttgaaat ttgaatgtaa ggttgtcata gctttgccac tcggaaatac agtcagtgag aaagaaaaaa 480 536 aactgtgtag tgttttttcc acaagtattt ggtgaattga ggttcttgaa atg gcg

gat cat caa tca gtg gtg tcg gta aac ttg ttt gtt gca ctt att tgc Asp His Gln Ser Val Val Ser Val Asn Leu Phe Val Ala Leu Ile Cys 20 25

gcg tgt att gtg atc ggt cat tta ttg gag gaa aac aga tgg atg aat Ala Cys Ile Val Ile Gly His Leu Leu Glu Glu Asn Arg Trp Met Asn 35 40 50

gag tcc ata act gcc ctt gtg att ggt agt tgc act gga gtc atc att Glu Ser Ile The Ala Leu Val Ile Gly Ser Cys Thr Gly Val Ile Ile 55 60 65

ttt gac ttt ggg act ctg ctg gga aag atg aac aac tta aca act tct Phe Asp Phe Gly Thr Leu Leu Gly Lys Met Asn Asn Leu Thr Thr Ser

| cta Leu | cta Leu | ata Ile | agt Ser 70 | gga Gly | gga Gly | aag Lys | aac Asn | tca Ser 75 | cat His | att Ile | tta Leu | gtg Val | ttc Phe 80 | agc Ser | gaa Glu | 776 |
|-------------------|-------------------|------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------|
| | | | | att Ile | | | | | | | | | | | | 824 |
| ttc Phe | cag Gln 100 | gtg Val | aaa Lys | aag Lys | aaa Lys | tca Ser 105 | ttc Phe | ttc Phe | cgc Arg | aat Asn | ttc Phe 110 | agt Ser | act Thr | atc Ile | atg Met | 872 |
| ctc Leu 115 | ttt Phe | ggg Gly | gca Ala | gtt Val | ggc Gly 120 | acc Thr | ttg Leu | ata Ile | tcg Ser | ttc Phe 125 | att Ile | att Ile | ata Ile | tca Ser | gcg Ala 130 | 920 |
| ggt Gly | gct Ala | att Ile | ggc Gly | att Ile 135 | ttc Phe | aag Lys | aaa Lys | atg Met | gat Asp 140 | att Ile | gga Gly | cac His | ctt Leu | gaa Glu 145 | att Ile | 968 |
| gga Gly | gat Asp | tac Tyr | ctt Leu 150 | gca Ala | att Ile | gga Gly | gca Ala | atc Ile 155 | ttt Phe | gct Ala | gca Ala | aca Thr | gat Asp 160 | tct Ser | gta Val | 1016 |
| | | | | gtg Val | | | | | | | | | | | | 1064 |
| cta Leu | gtg Val 180 | ttt Phe | gga Gly | gaa Glu | ggt Gly | gtt Val 185 | gtg Val | aat Asn | gat Asp | gcc Ala | aca Thr 190 | tct Ser | gta Val | gtg Val | ctg Leu | 1112 |
| | | | | cag Gln | | | | | | | | | | | | 1160 |
| gct Ala | ctg Leu | caa Gln | tta Leu | att Ile 215 | gga Gly | aac Asn | ttt Phe | cta Leu | tac Tyr 220 | ttg Leu | ttt Phe | gcc Ala | tcg Ser 225 | agc Ser | act Thr | 1208 |
| ttc Phe | cta Leu | ggg Gly | gtt Val 230 | gct Ala | gtt Val | ggc Gly | cta Leu 235 | cta Leu | agt Ser | gcc Ala | ttt Phe | ata Ile 240 | att Ile | aag Lys | aaa Lys | 1256 |
| | | | | agg Arg | | | | | | | | | | | | 1304 |
| ctc Leu | atg Met 260 | gcg Ala | tac Tyr | cta Leu | tca Ser | tac Tyr 265 | atg Met | ctt Leu | gct Ala | gaa Glu | tta Leu 270 | ttc Phe | tat Tyr | tta Leu | agt Ser | 1352 |
| gga Gly 275 | atc Ile | ctc Leu | act Thr | gtg Val | ttt Phe 280 | ttc Phe | tgt Cys | ggg Gly | atc Ile | gtg Val 285 | atg Met | tct Ser | cac His | tat Tyr | acc Thr 290 | 1400 |
| tgg | cat | aat | gtg | act | gag | agc | tca | aga | gtc | act | acc | aag | cac | acg | ttt | 1.448 |

| Trp | His | Asn | Val | Thr 295 | Glu | Ser | Ser | Arg | Val 300 | Thr | Thr | Lys | His | Thr 305 | Phe | |
|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|------|
| gct Ala | aca Thr | tta Leu | tca Ser 310 | ttt Phe | att Ile | gct Ala | gaa Glu | ata Ile 315 | ttc Phe | ata Ile | ttc Phe | ctt Leu | tat Tyr 320 | gtt Val | ggt Gly | 1496 |
| atg Met | gat Asp | gct Ala 325 | ttg Leu | gac Asp | att Ile | gag Glu | aag Lys 330 | tgg Trp | aag Lys | ttt Phe | gta Val | agc Ser 335 | gac Asp | agc Ser | ccc Pro | 1544 |
| gga Gly | aca Thr 340 | tca Ser | att Ile | aag Lys | gtc Val | agc Ser 345 | tca Ser | att Ile | ctg Leu | cta Leu | ggt Gly 350 | ctt Leu | gtt Val | ttg Leu | gtt Val | 1592 |
| gga Gly 355 | agg Arg | gga Gly | gcc Ala | ttt Phe | gtt Val 360 | ttc Phe | ccc Pro | ttg Leu | tca Ser | ttc Phe 365 | ttg Leu | tcc Ser | aac Asn | ttg Leu | acc Thr 370 | 1640 |
| aag Lys | aaa Lys | aat Asn | cct Pro | gag Glu 375 | gac Asp | aag Lys | att Ile | agc Ser | ttt Phe 380 | aac Asn | cag Gln | cag Gln | gtt Val | aca Thr 385 | ata Ile | 1688 |
| tgg Trp | tgg Trp | gct Ala | ggg Gly 390 | ctt Leu | atg Met | cga Arg | ggt Gly | gct Ala 395 | gtt Val | tct Ser | atg Met | gcc Ala | ctt Leu 400 | gct Ala | tat Tyr | 1736 |
| aat Asn | cag Gln | ttt Phe 405 | acc Thr | agg Arg | gga Gly | ggt Gly | cat His 410 | act Thr | cag Gln | tta Leu | cgt Arg | gcc Ala 415 | aat Asn | gca Ala | ata Ile | 1784 |
| atg Met | atc Ile 420 | acg Thr | agt Ser | act Thr 425 | atc Ile | act Thr | gtt Val | gtc Val | ctt Leu | ttc Phe | agc Ser 430 | aca Thr | gtg Val | gta Val | ttt Phe | 1832 |
| ggg Gly 435 | ttg Leu | atg Met | aca Thr | aaa Lys | cct Pro 440 | tta Leu | att Ile | cta Leu | tta Leu | ttg Leu 445 | Leu | ccc Pro | tca Ser | caa Gln | aaa Lys 450 | 1880 |
| cac His | ttg Leu | atc Ile | aga Arg | atg Met 455 | atc Ile | tcc Ser | tct Ser | gaa Glu | ccg Pro 460 | atg Met | act Thr | cca Pro | aaa Lys | tcc Ser 465 | ttc Phe | 1928 |
| att | gtg Val | cca Pro | ctt Leu 470 | ctt Leu | gac Asp | agc Ser | aca Thr | caa Gln 475 | gac Asp | tca Ser | gaa Glu | gct Ala | gat Asp 480 | ctg Leu | ggc Gly | 1976 |
| cga Arg | cat His | gta Val 485 | ccc Pro | cgt Arg | ccc Pro | cac His | agt Ser 490 | ttg Leu | cgg Arg | atg Met | ctc Leu | ctg Leu 495 | Ser | acc Thr | cca Pro | 2024 |
| tct Ser | cac His 500 | Thr | gta Val | cat His | tac Tyr 505 | Tyr | tgg Trp | aga Arg | aaa Lys | ttt Phe | gac Asp 510 | Asn | gca Ala | ttc Phe | atg Met | 2072 |
| cgt Arg | cct | gtt Val | ttc Phe | ggt Gly | gga Gly | cga Arg | ggt Gly | ttt Phe | gta Val | cct Pro | ttt Phe | gtt Val | cca Pro | gga Gly | tca Ser | 2120 |

515 530 cet act gaa eeg gte gaa eeg ace gaa eea aga eea gee gaa tea aga 2168 Pro Thr Glu Pro Val Glu Pro Thr Glu Pro Arg Pro Ala Glu Ser Arg cca acc gaa cca act gat gag tgattacact gatggagatg caggttgcac 2219 Pro Thr Glu Pro Thr Asp Glu 550 taaagtccca ctggccttgg agaaggacga aggcagtttt ttgggtttga ggttttgttt 2279 actqttaata qttttcqaat qtggttaaaa aagggttgtc tagtttttat atataggtcg 2339 cagatacgta atttcagctc agttcccgag gtgaacccct tagaggtttt cttcctgacg 2399 gtttttcttc ttttttgtaa tttatcaaaa acaccaaatg ggtgtatatt ctttaagctt 2459 gtagettaat tacettataa geatgtggta gegttegtgt aatatgtaaa attteeattg 2519 2553 ccagaaaaga aacttccata caatatttct gccg <210> 17 <211> 553 <212> PRT <213> Nierembergia hybrida <220> <221> peptide (1)..(553) <223> Amino acid sequence of protein regulating the pH of vacuoles <400> 17 Met Ala Phe Asp Phe Gly Thr Leu Leu Gly Lys Met Asn Asn Leu Thr Thr Ser Asp His Gln Ser Val Val Ser Val Asn Leu Phe Val Ala Leu Ile Cys Ala Cys Ile Val Ile Gly His Leu Leu Glu Glu Asn Arg Trp Met Asn Glu Ser Ile Thr Ala Leu Val Ile Gly Ser Cys Thr Gly Val Ile Ile Leu Leu Ile Ser Gly Gly Lys Asn Ser His Ile Leu Val Phe 65 Ser Glu Asp Leu Phe Phe Ile Tyr Leu Leu Pro Pro Ile Ile Phe Asn Ala Gly Phe Gln Val Lys Lys Ser Phe Phe Arg Asn Phe Ser Thr 100 Ile Met Leu Phe Gly Ala Val Gly Thr Leu Ile Ser Phe Ile Ile Ile

120 125 Ser Ala Gly Ala Ile Gly Ile Phe Lys Lys Met Asp Ile Gly His Leu Glu Ile Gly Asp Tyr Leu Ala Ile Gly Ala Ile Phe Ala Ala Thr Asp 155 Ser Val Cys Thr Leu Gln Val Leu Asn Gln Glu Glu Thr Pro Leu Leu 170 Tyr Ser Leu Val Phe Gly Glu Gly Val Val Asn Asp Ala Thr Ser Val 185 Val Leu Phe Asn Ala Val Gln Asn Phe Asp Leu Ser His Ile Ser Thr Gly Lys Ala Leu Gln Leu Ile Gly Asn Phe Leu Tyr Leu Phe Ala Ser Ser Thr Phe Leu Gly Val Ala Val Gly Leu Leu Ser Ala Phe Ile Ile Lys Lys Leu Tyr Phe Gly Arg His Ser Thr Asp Arg Glu Val Ala Ile Met Ile Leu Met Ala Tyr Leu Ser Tyr Met Leu Ala Glu Leu Phe Tyr Leu Ser Gly Ile Leu Thr Val Phe Phe Cys Gly Ile Val Met Ser His 280 Tyr Thr Trp His Asn Val Thr Glu Ser Ser Arg Val Thr Thr Lys His Thr Phe Ala Thr Leu Ser Phe Ile Ala Glu Ile Phe Ile Phe Leu Tyr Val Gly Met Asp Ala Leu Asp Ile Glu Lys Trp Lys Phe Val Ser Asp Ser Pro Gly Thr Ser Ile Lys Val Ser Ser Ile Leu Leu Gly Leu Val Leu Val Gly Arg Gly Ala Phe Val Phe Pro Leu Ser Phe Leu Ser Asn Leu Thr Lys Lys Asn Pro Glu Asp Lys Ile Ser Phe Asn Gln Gln Val Thr Ile Trp Trp Ala Gly Leu Met Arg Gly Ala Val Ser Met Ala Leu Ala Tyr Asn Gln Phe Thr Arg Gly Gly His Thr Gln Leu Arg Ala Asn

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| Ala | Ile | Met | Ile 420 | Thr | Ser | Thr | Ile | Thr 425 | Val | Val | Leu | Phe | Ser 430 | Thr | Val | |
|------------------------------|--------------------|---------------------------|--------------|-------------|------------|------------|------------|------------|------------|-----------|------------|------------|------------|------------|-----------|-----|
| Val | Phe | Gly 435 | Leu | Met | Thr | Lys | Pro 440 | Leu | Ile | Leu | Leu | Leu 445 | Leu | Pro | Ser | |
| Gln | Lys 450 | His | Leu | Ile | Arg | Met 455 | Ile | Ser | Ser | Glu | Pro 460 | Met | Thr | Pro | Lys | |
| Ser 465 | Phe | Ile | Val | | Leu 70 | Leu | Asp | Ser | | Gln 75 | Asp | Ser | Glu | | Asp 30 | |
| Leu | Gly | Arg | His | Val 485 | Pro | Arg | Pro | His | Ser 490 | Leu | Arg | Met | Leu | Leu 495 | Ser | |
| Thr | Pro | Ser | His 500 | Thr | Val | His | Tyr | Tyr 505 | Trp | Arg | Lys | Phe | Asp 510 | Asn | Ala | |
| Phe | Met | Arg 515 | Pro | Val | Phe | Gly | Gly 520 | Arg | Gly | Phe | Val | Pro 525 | Phe | Val | Pro | |
| Gly | Ser 530 | Pro | Thr | Glu | Pro | Val 535 | Glu | Pro | Thr | Glu | Pro 540 | Arg | Pro | Ala | Glu | |
| Ser 545 | Arg | Pro | Thr | Glu | Pro 550 | Thr | Asp | Glu | | | | | | | | |
| <210 <211 <211 <211 | 1> . 2> . | 18 2361 DNA Tore | nia : | hybr: | ida | | | | | | | | | | | |
| <22 <22 <22 <22 | 1> : 2> 3> : | misc (1). Nucl | .(23 eoti | 61) de s | | nce | of Di | NA e | ncod | ing | for | prot | ein | regu | lating | the |
| | 0> 1 ggag | | ccga | gctg | ca g | catc | acct: | t gc | ttat | gtaa | gct | ttaa | aag | tatc | agaatt | 60 |
| gaa | tatc | gac | cact | ggaa | ag t | gttt | tagg | a ct | tgga | ttot | tat | ctat | tga | gctt | gtttga | 120 |
| agg | tgaa | aaa | aggc | toga | tc t | cgtt | cctc | t at | agtt | ggtt | ttc | tgga | gtt | gcaa | gcgact | 180 |
| cta | ctcg | gaa | tctc | tttc | cg c | ctta | ttgg | a ag | ctct | gctt | tac | taaa | aaa | agtt | tgtctt | 240 |
| ttt | atct | ctg. | attc | atca | ta a | aatc | tgcg | g ga | gatt | caga | agc | ggag | atc | tggt | gcccag | 300 |
| agc | agga | gtt | tcaa | cttt | ga g | cccg | ttta | t at | ttat | aaac | aaa | ttcc | gag | tcca | aagatt | 360 |
| gaa | cttt | gaa | ataa | tcaa | at a | atca | agca. | a gc | | | | | | tct Ser | | 413 |

att aag cta geg gea agt gaa act gae aat ttg tgg age tet ggt eac 461

| Ile | Lys | Leu | Ala 10 | Ala | Ser | Glu | Thr | Asp 15 | Asn | Leu | Trp | Ser 20 | Ser | Gly | His | |
|------------|------------|------------|-------------------|-------------------|------------|------------|------------|-------------------|------------|------------|------------|------------|-------------------|------------|------------|------|
| | | | | gct Ala | | | | | | | | | | | | 509 |
| | | | | cat His | | | | | | | | | | | | 557 |
| | | | | ata Ile | | | | | | | | | | | | 605 |
| | | | | aaa Lys 75 | | | | | | | | | | | | 653 |
| ttc Phe | ttc Phe | atc Ile | tat Tyr 90 | gcg Ala | ctg Leu | cca Pro | cca Pro | atc Ile 95 | att Ile | ttt Phe | aat Asn | gcg Ala | ggg Gly 100 | ttc Phe | caa Gln | 701 |
| | | | | tca Ser | | | | | | | | | | | | 749 |
| | | | | acc Thr | | | | | | | | | | | | 797 |
| | | | | ccc Pro | | | | | | | | | | | | 845 |
| | | | | gct Ala 155 | | | | | | | | | | | | 893 |
| cag Gln | gtg Val | cta Leu | agc Ser 170 | cag Gln | gac Asp | gaa Glu | aca Thr | cca Pro 175 | ctg Leu | ttg Leu | tac Tyr | agt Ser | cta Leu 180 | gtg Val | ttt Phe | 941 |
| | | | | gta Val | | | | | | | | | | | | 989 |
| | | | | gac Asp | | | | | | | | | | | | 1037 |
| | | | | ttc Phe 220 | | | | | | | | | | | | 1085 |
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| Val | Leu | Thr | Gly | Leu 235 | Leu | Ser | Ala | Tyr | Ile 240 | Ile | Lys | Lys | Leu | Tyr 245 | Phe | |
|------------|------------|------------|-------------------|-------------------|------------|------------|------------|-------------------|-------------------|------------|------------|------------|-------------------|-------------------|------------|------|
| gga Gly | agg Arg | cac His | tcc Ser 250 | act Thr | gat Asp | cgc Arg | gag Glu | gtt Val 255 | gcc Ala | ata Ile | atg Met | ata Ile | ctc Leu 260 | atg Met | gct Ala | 1181 |
| | | | | atg Met | | | | | | | | | | | | 1229 |
| | | | | tgt Cys | | | | | | | | | | | | 1277 |
| | | | | tca Ser | | | | | | | | | | | | 1325 |
| tca Ser | ttt Phe | gtt Val | gct Ala | gaa Glu 315 | ata Ile | ttt Phe | ata Ile | ttt Phe | ctg Leu 320 | tat Tyr | gtt Val | ggc Gly | atg Met | gat Asp 325 | gct Ala | 1373 |
| | | | | aaa Lys | | | | | | | | | | | | 1421 |
| | | | | gca Ala | | | | | | | | | | | | 1469 |
| | | | | cct Pro | | | | | | | | | | | | 1517 |
| | | | | atc Ile | | | | | | | | | | | | 1565 |
| | | | | gga Gly 395 | | | | | | | | | | | | 1613 |
| | | | | ctc Leu | | | | | | | | | | | | 1661 |
| | | | | att Ile | | | | | | | | | | | | 1709 |
| | | | | atc Ile | | | | | | | | | | | | 1757 |
| | | | | gaa Glu | | | | | | | | | | | | 1805 |

| 455 | 460 | 405 | 470 |
|---|---|---|--|
| | | 465 | 470 |
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| caa act tca caa Gln Thr Ser Gln 490 | Gly Gly Glu Pro | gtt gct cga ccg agc a Val Ala Arg Pro Ser 8 495 | ago ota ogo 1901 Ser Leu Arg 500 |
| atg tta ctc aca Met Leu Leu Thr 505 | aag ccc act cat Lys Pro Thr His 510 | acg gtg cac tat tat t Thr Val His Tyr Tyr 1 515 | gg aga aaa 1949 Prp Arg Lys |
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| cca tat gtt ccc Pro Tyr Val Pro 535 | ggt tca ccg act Gly Ser Pro Thr 540 | gaa oga ago gtt ogo a Glu Arg Ser Val Arg <i>F</i> 545 | ac tgg gaa 2045 sn Trp Glu 550 |
| gaa gag acc aaa Glu Glu Thr Lys | cag taaaaagatt t Gln 555 | tettgtgtg aatgatggtg | aagagattag 2100 |
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| | | atatttttgt tagaaacto | |
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| <220> <221> peptide <222> (1)(555) <223> Amino aci | | otein regulating the | pH of vacuoles |
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| Leu Trp Ser Ser 20 | Gly His Gly Ser | Val Val Ala Ile Thr I 25 | eu Phe Val 30 |
| Thr Leu Leu Cys 35 | Thr Cys Ile Val : | (le Gly His Leu Leu G 45 | lu Glu Asn |

FILLER CONTROL CONTROL OF THE CONTRO

Arg Trp Met Asn Glu Ser Ile Ile Ala Leu Ile Ile Gly Leu Ala Thr Gly Val Ile Ile Leu Leu Ile Ser Gly Gly Lys Ser Ser His Leu Leu Val Phe Ser Glu Asp Leu Phe Phe Ile Tyr Ala Leu Pro Pro Ile Ile 85 90 95 Phe Asn Ala Gly Phe Gln Val Lys Lys Ser Phe Phe Arg Asn Phe Ala Thr Ile Met Met Phe Gly Ala Val Gly Thr Leu Ile Ser Phe Ile Ile Ile Ser Leu Gly Thr Ile Ala Phe Phe Pro Lys Met Asn Met Arg Leu Gly Val Gly Asp Tyr Leu Ala Ile Gly Ala Ile Phe Ala Ala Thr Asp Ser Val Cys Thr Leu Gln Val Leu Ser Gln Asp Glu Thr Pro Leu 165 170 175 Leu Tyr Ser Leu Val Phe Gly Glu Gly Val Val Asn Asp Ala Thr Ser Val Val Leu Phe Asn Ala Val Gln Asn Phe Asp Leu Pro His Met Ser Thr Ala Lys Ala Phe Glu Leu Val Gly Asn Phe Phe Tyr Leu Phe Ala Thr Ser Thr Val Leu Gly Val Leu Thr Gly Leu Leu Ser Ala Tyr Ile 225 230 240 Ile Lys Lys Leu Tyr Phe Gly Arg His Ser Thr Asp Arg Glu Val Ala Ile Met Ile Leu Met Ala Tyr Leu Ser Tyr Met Leu Ala Glu Leu Phe

Asp Leu Ser Gly Ile Leu Thr Val Phe Phe Cys Gly Ile Val Met Ser

His Tyr Thr Trp His Asn Val Thr Glu Asn Ser Arg Val Thr Thr Lys

His Thr Phe Ala Thr Leu Ser Phe Val Ala Glu Ile Phe Ile Phe Leu

Tyr Val Gly Met Asp Ala Leu Asp Ile Glu Lys Trp Arg Phe Val Ser

Gly Ser Met Thr Thr Ser Ala Ala Val Ser Ala Thr Leu Leu Gly Leu 345

Val Leu Leu Ser Arg Ala Ala Phe Val Phe Pro Leu Ser Phe Leu Ser Asn Leu Ala Lys Lys Ser Pro Leu Glu Lys Ile Ser Leu Arg Gln Gln Ile Ile Ile Trp Trp Ala Gly Leu Met Arg Gly Ala Val Ser Met Ala Leu Ala Tyr Lys Gln Phe Thr Arg Glu Gly Leu Thr Val Glu Arg Glu Asn Ala Ile Phe Ile Thr Ser Thr Ile Thr Ile Val Leu Phe Ser Thr Val Val Phe Gly Leu Met Thr Lys Pro Leu Ile Asn Leu Leu Ile Pro 435 Ser Pro Lys Leu Asn Arg Ser Val Ser Ser Glu Pro Leu Thr Pro Asn Ser Ile Thr Ile Pro Leu Leu Gly Glu Ser Gln Asp Ser Val Ala Glu Leu Phe Ser Ile Arg Gly Gln Thr Ser Gln Gly Gly Glu Pro Val Ala Arg Pro Ser Ser Leu Arg Met Leu Leu Thr Lys Pro Thr His Thr Val His Tyr Tyr Trp Arg Lys Phe Asp Asn Ala Phe Met Arg Pro Val Phe Gly Gly Arg Gly Phe Val Pro Tyr Val Pro Gly Ser Pro Thr Glu Arg 530 540 Ser Val Arg Asn Trp Glu Glu Glu Thr Lys Gln <210> 20 <211> 6298 <212> DNA <213> Ipomea nil <220> <221> misc_feature <222> (1)..(6298) <223> Nucleotide sequence of promoter region of gene encoding for protein regulating the pH of vacuoles

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